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Responses of Stream Invertebrates to an Ashpit Effluent, Wisconsin Power Plant Impact Study

Wisconsin Univ.-Madison

Prepared for

Environmental Research Lab.-Duluth, MN

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Duluth MN 55804

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Responses of Stream Invertebrates to an Ashpit Effluent

Wisconsin Power Plant Impact Study

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Fly ash from the 527-MW coal-fired Columbia Generating Station Unit I (Columbia Co., Wisconsin) is discharged as a slurry into an adjacent ashpit. Water from the ashpit is pumped to a ditch that joins the ashpit drain and Rocky Run Creek before they reach the Wisconsin River. Habitat alterations have been noted as relatively minor changes in water quality parameters (e.g., alkalinity, hardness, pH, and turbidity), as increased amounts of some dissolved trace elements (Cr, Ba, Al, Cd, and Cu), and as the precipitation of trace elements (Al, Ba, and Cr) into a floc that coats the stream bottoms. The ashpit drain became an unsuitable habitat for aquatic invertebrates after Columbia I began operating.

The conductivity of the effluent increased in January 1977 when sodium bicarbonate was first used to increase the efficiency of the electrostatic precipitators. Since then conductivity measurements have indicated effluent concentration at distances downstream from the generating station.

Rocky Run Creek is still a suitable habitat for many aquatic invertebrates, but evidence of sublethal stresses and habitat avoidance exists. The major effect of Columbia I on aquatic invertebrates is hypothesized to be continued habitat alteration and, in particular, reduced substrate quality and avoidance of unpreferred habitat. The susceptibility of early life stages of crustaceans to the ash effluent may also be important. Acute toxicity to adult forms is unimportant.

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RESPONSES OF STREAM INVERTEBRATES TO AN ASHPIT EFFLUENT

Wisconsin Power Plant Impact Study

by

John J. Magnuson
Anne M. Forbes
Dorothy M. Harrell
Judy D. Schwarzmeier
Institute for Environmental Studies
University of Wisconsin-Madison

Grant No. R803971

Project Officer

Gary E. Glass
Environmental Research Laboratory-Duluth
Duluth, Minnesota

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Madison Gas and Electric Company, WE
Wisconsin Public Service Corporation, Grn Ba
Wisconsin Public Service Commission, Madison
and Wisconsin Department of Natural Resources Madison

ENVIRONMENTAL RESEARCH LABORATORY-DULUTH
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MINNESOTA 55804

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FOREWORD

The U.S. Environmental Protection Agency (EPA) was designed to coordinate our country's efforts toward protecting and improving the environment. This extremely complex task requires continuous research in a multitude of scientific and technical areas. Such research is necessary to monitor changes in the environment, to discover relationships within that environment, to determine health standards, and to eliminate potentially hazardous effects.

One project, which the EPA is supporting through its Environmental Research Laboratory in Duluth, Minnesota, is the study "The Impacts of Coal-Fired Power Plants on the Environment. This interdisciplinary study, centered mainly around the Columbia Generating Station near Portage, Wis., involves investigators and experiments from many academic departments at the University of Wisconsin and is being carried out by the Environmental Monitoring and Data Acquisition Group of the Institute for Environmental Studies at the University of Wisconsin-Madison. Several utilities and State agencies are cooperating in the study: Wisconsin Power and Light Company, Madison Gas and Electric Company, Wisconsin Public Service Corporation, Wisconsin Public Service Commission, and Wisconsin Department of Natural Resources.

During the next year reports from this study will be published as a series within the EPA Ecological Research Series. These reports will include topics related to chemical constituents, chemical transport mechanisms, biological effects, social and economic effects, and integration and synthesis.

Since Columbia I began operating the ashpit drain has become an unsuitable habitat for aquatic invertebrates. Upstream-downstream differences in invertebrate communities in Rocky Run Creek were observed when ash effluent concentration was high. The effects of Columbia I were undetectable from natural variation in the Wisconsin River. The major effect of Columbia I on aquatic invertebrates is hypothesized to be continued habitat alteration and, in particular, reduced substrate quality and avoidance of unpreferred habitat.

Norbert A. Jaworski
Director
Environmental Research Laboratory
Duluth, Minnesota

ABSTRACT

Fly ash from the 527-MW coal-fired Columbia Generating Station Unit I (Columbia Co., Wisconsin) is discharged as a slurry into an adjacent ashpit. Water from the ashpit is pumped to a ditch that joins the ashpit drain and Rocky Run Creek before they reach the Wisconsin River. Habitat alterations have been noted as relatively minor changes in water quality parameters (e.g., alkalinity, hardness, pH, and turbidity), as increased amounts of some dissolved trace elements (Cr, Ba, Al, Cd, and Cu), and as the precipitation of trace elements (Al, Ba, and Cr) into a floc that coats the stream bottoms.

The ashpit drain became an unsuitable habitat for aquatic invertebrates after Columbia I began operating. Upstream-downstream differences in invertebrate communities in Rocky Run Creek were observed when ash effluent concentration was high. The effects of Columbia I were undetectable from natural variation in the Wisconsin River. The collection of data in only 1 preoperational year severely limited the analysis of generating station impact.

Crayfish caged downstream from the ash effluent survived at the same rate as those caged at upstream control sites, but they contained higher levels of metals (Cr, Ba, Zn, Se, and Fe) and had lower metabolic rates. Crayfish fed food containing Cr in the laboratory accumulated less than 3% of the amount ingested. However, Cr in food may be an important factor affecting invertebrate populations at the Columbia I site because its concentration on particulates was high.

Survival of winter-generation *Asellus racovitzai* was similar when exposed to control and ash-effluent water and to control food and food exposed to ash effluent. Poor late-winter condition of the isopods precluded detection of any sublethal effects. However, young-of-the-year instars of *Gammarus pseudolimnaeus* were more sensitive to the ash effleunt than were adults.

The conductivity of the effluent increased in January 1977 when sodium bicarbonate was first used to increase the efficiency of the electrostatic precipitators. Since then conductivity measurements have indicated effluent concentration at distances downstream from the generating station. Thresholds for field and laboratory responses to the effluent, as measured by conductivity, were estimated at 800 to 1,459 μmhos/cm and averaged about 1,100 μmhos. The annual record of conductivity was used to observe how often the threshold was exceeded.

Rocky Run Creek is still a suitable habitat for many aquatic invertebrates, but evidence of sublethal stresses and habitat avoidance exists. The major effect of Columbia I on aquatic invertebrates is hypothesized to be continued habitat alteration and, in particular, reduced substrate quality and avoidance of unpreferred habitat. The susceptibility of early life stages of crustaceans to the ash effluent may also be important. Acute toxicity to adult forms is unimportant.

This report was prepared with the cooperation of faculty and graduate students in the Laboratory of Limnology at the University of Wisconsin-Madison.

Most of the funding for the research reported here was provided by the U.S. Environmental Protection Agency (U.S. EPA). Funds also were granted by the University of Wisconsin-Madison, Wisconsin Power and Light Company, Madison Gas and Electric Company, the Wisconsin Public Service Corporation, and the Wisconsin Public Service Commission. This report is submitted toward fulfillment of Grant No. R803971 by the Environmental Monitoring and Data Acquisition Group, Institute for Environmental Studies, University of Wisconsin-Madison, under the partial sponsorship of the U.S. EPA. The report covers the period July 1, 1975 to July 1, 1978 and work was completed as of April 10, 1979.

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SECTION 1

INTRODUCTION

PURPOSES OF THIS STUDY

The original objective of this project was to study the impact of the Columbia Electric Generating Station on aquatic macroinvertebrates in the Wisconsin River, Rocky Run Creek, and the ashpit drain. Broad spatial and temporal changes in invertebrate communities were observed using multivariate analyses. Samples were collected from artificial substrates at points upstream and downstream from the generating station 1 yr before (1974) and 3 yr after (1975, 1976, and 1977) operation began. This work is documented in Section 3 of this paper, "Effects on Community Structure on Macroinvertebrates."

The changes in the physical layout and chemical inputs at the Columbia site have altered the aquatic habitats. Of special interest to this project were the effects of habitat alterations on aquatic invertebrates living downstream from the ash effluent discharge. Negative effects of the effluent could be considered in light of acute toxicity, sublethal toxicity, avoidance of less favorable habitat, or a combination of the above. Changes in the trace element content of organisms living in the ashpit drain were measured by Helmke et al.

Midway through the study, several more specific problems were focused on: (1) Completing the determination of trace-element levels in aquatic organisms upstream and downstream from the ash effluent (Trace Elements Subproject) and gathering data on the biological significance of the changes observed (Aquatic Invertebrates Subproject); (2) evaluating the effects of the ashpit effluent as a whole on local invertebrate fauna using a combination of laboratory and field experiments (Aquatic Invertebrates Subproject) (supporting data on the effluent contents came from the Aquatic Chemistry and Trace Elements subprojects); and (3) continuing to monitor aquatic invertebrate communities in Rocky Run Creek upstream and downstream of the generating station.

Five studies were initiated to satisfy these objectives: (1) Exposure of crayfish in cages upstream and downstream from the ash effluent; (2) follow-up measurements of metabolic rates and trace element body burdens for crayfish caged in the field; (3) a laboratory feeding study of the uptake and effects of chromium on crayfish; (4) laboratory exposures of amphipods and isopods to the ash effluent for data on survival, growth, and reproductive success; (5) an examination of spatial and temporal variability in life histories of mayfly nymphs collected in the study area. The materials, methods, results, and discussion of these five studies are presented in Section 4, "Effects on Individual Organisms."

Overall conclusions for this paper are given in Section 2, and the bibliography is presented in Section 5. The appendices contain literature reviews: Appendix A, entrainment from cooling lake intake; Appendix B, acid rain; Appendix C, alternative disposal of fly ash; Appendix D, effects of chromium and other heavy metals. Appendices E and F contain the supporting data analysis from the crayfish experiments.

THE GENERATING STATION SITE

The Columbia Generating Station Unit I is a 527-MW coal-fired electric generating station located on the eastern floodplain of the Wisconsin River, 5 km south of Portage, Wisconsin (Figure 1). The generating station building is 73 m (240 ft) high with a 150-m (500-ft) boiler chimney equipped with two electrostatic precipitators. Construction of Columbia I began in 1971; operation began in April 1975. Columbia II, a second unit of similar size, with a 195-m (650-ft) stack, cooling towers, and sulfur-removal scrubbers, began operating in spring 1978.

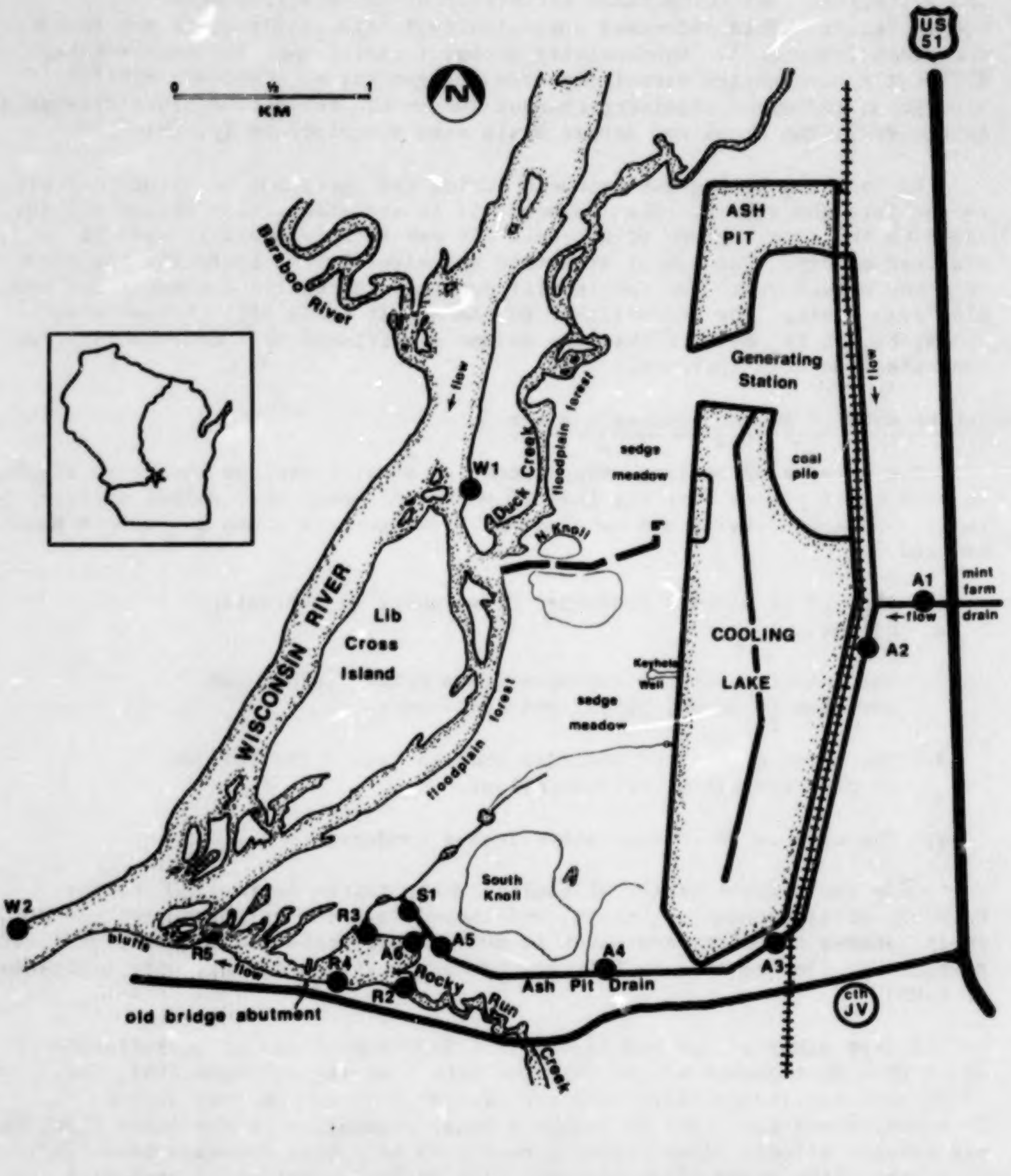
The 2,726-acre site of these dual generating stations covers a range of plant and animal communities, including aquatic, wetland, and forested areas. The installation has permanently altered 1,100 acres which includes a 500-acre cooling lake, 70-acre ash basin, coal-handling facilities, roads, and various other structures. The cooling lake, designed to recycle the thermal effluent from the generating station, was built on 500 acres of native wetlands. Water from the Wisconsin River was used to fill the cooling lake and is still pumped almost continuously into the lake to make up for evaporation and leakage losses.

GENERATING STATION OPERATION AND ADJACENT AQUATIC HABITATS

Fly Ash

Columbia I burns about 5,000 tons/day of low-sulfur pulverized coal from Colstrip, Montana, with a typical ash content of 7 to 8%. The high energy electrostatic precipitators installed to reduce particulate emissions collect approximately 98% of this "fly ash" residue and discharge it as a slurry with cooling pond water into the ashpit adjacent to the plant. Water entering the ashpit flows through a series of lagoons where the ash particles settle out. The water is then pumped to the ashpit drain and eventually combines with the water of Rocky Run Creek (Figure 1).

The chemistry of the ashpit has been studied (Andren et al. 1977). The metal oxides composing the major reactive portions of the ash result in a water pH of 10 to 11. Since Wisconsin water quality standards prohibit the release of water > pH 8, sulfuric acid is added before the ash effluent is discharged. Adding acid causes elements such as Ba, Al, and Cr to precipitate into a floc that coats the bottom of the ashpit drain and is carried with the current into Rocky Run Creek and the Wisconsin River.



0
½
1
KM
N
US 51
Baraboo River
flow
W1
Duck Creek
floodplain forest
sedge meadow
ASH PIT
Generating Station
flow
coal pile
H. Knoll
A1
mint farm drain
flow
A2
COOLING LAKE
Keyhole Well
sedge meadow
WISCONSIN RIVER
Lib Cross Island
floodplain forest
South Knoll
S1
R3
A5
A6
W2
bluffs
R5
flow
R4
R2
Rocky Run Creek
A4
Ash Pit Drain
A3
cth JV
old bridge abutment

Figure 1. Location of invertebrate sampling stations in streams near the Columbia Generating Station.

Beginning in January 1977, sodium bicarbonate was routinely added to the pulverized coal to increase efficiency of the electrostatic precipitators. This increased conductivity in the ashpit drain and Rocky Run Creek (Figure 2). Conductivity became a useful tool for measuring ash effluent concentration downstream from the generating station. Habitat alteration and water chemistry changes due to the ash effluent are discussed in the Rocky Run Creek and Ashpit Drain site descriptions (p. 8-12).

Fly ash and bottom ash produced during the operation of Columbia I are pumped into the ashpit. When Columbia II is operating, only bottom ash is added to the total volume of ash; all fly ash from Columbia II must be disposed of dry. The ashpit structure was altered in preparation for the handling of dry ash. The ashpit will continue to receive demineralizer and blow-down waste. How the effluent to the ashpit drain will change is not known, but it is possible that the volume of effluent will decrease and its concentration will increase.

Intake Water from the Wisconsin River

The effects of cooling water intake on aquatic systems have been studied at many power plants over the last 20 years. Although the studies differed in their approach, detail, and conclusions, four general areas of concern have emerged:

1. Removal of animals suspended or swimming in the water column.
2. Mechanical injury by impingement on intake screens or abrasion in pumps, pipes, and condensers.
3. The toxic effects of biocides used to reduce the fouling of pipe systems by microorganisms.
4. The effects of thermal shock during condenser passage.

Only the removal aspect of cooling water intake is relevant to the Columbia site; mechanical, toxic, and thermal aspects of entrainment do not apply because the entrained water is not directly returned to the river. The total river flow removed at Columbia presently averages 0.3%, with a maximum of 1.08%.

A 1-yr study of egg and larval fish entrainment and of juvenile and adult fish impingement at the Columbia site (Swanson Environmental, Inc. 1977) reported insignificant numbers of fish losses. As long as the Columbia intake continues to remove a small percentage of the river flow, no measureable effects of entrainment on the river system are expected. An exception might occur if an organism with patchy distribution becomes concentrated near the intake and a significant portion of one year-class (i.e., walleye larvae) becomes entrained. Aside from removing organisms from the Wisconsin River, the usual entrainment effects (mechanical, toxic, and thermal) do not occur at the Columbia station.

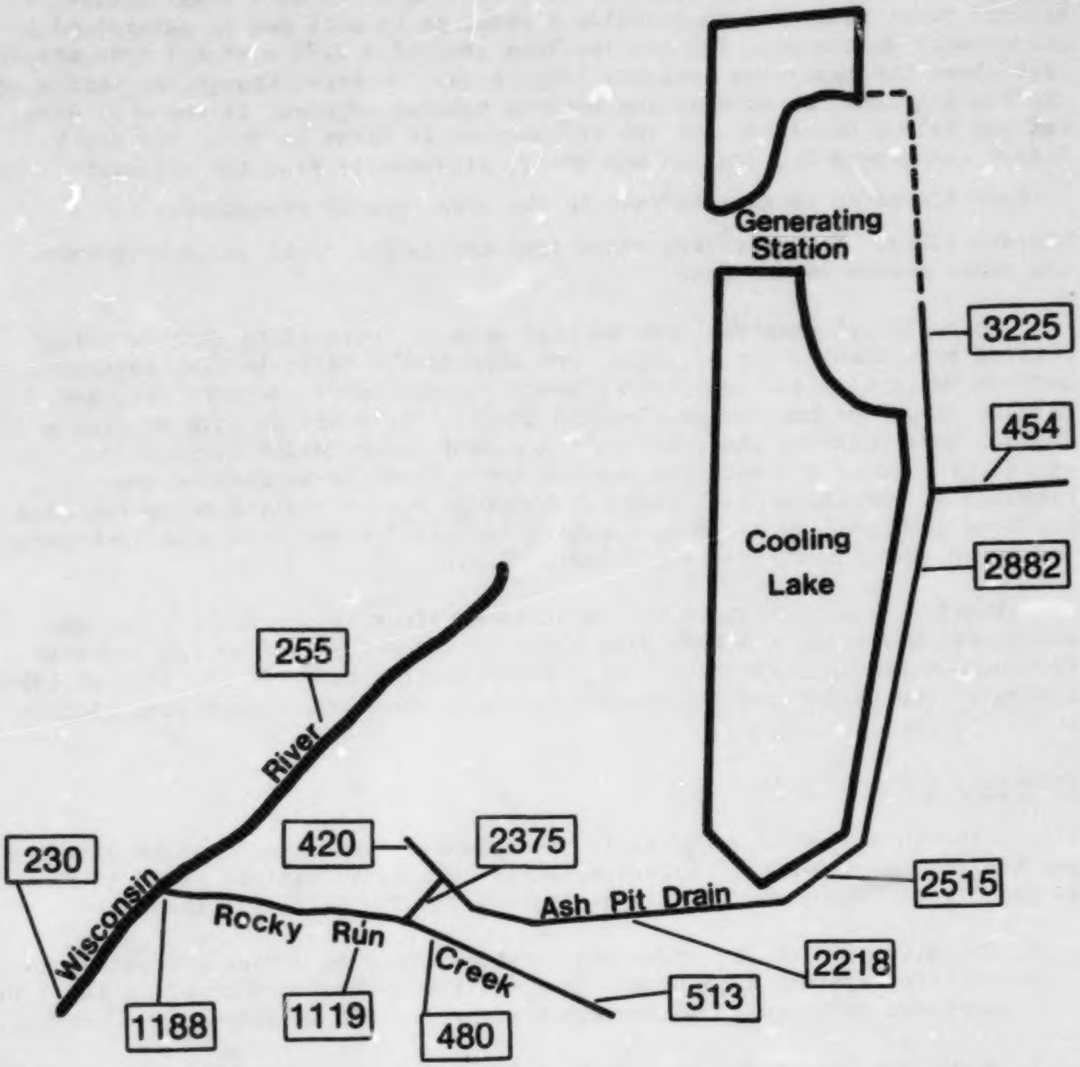

Generating Station
Cooling Lake
3225
454
2882
2515
255
River
Wisconsin
230
420
2375
Ash Pit Drain
Rocky Run Creek
2218
513
1188
1119
480

Figure 2. Conductivity (μmhos/cm) gradient in streams adjacent to the Columbia Generating Station in September 1977.

Leakage from the Cooling Lake and Ash Basin

The cooling lake was built by constructing dikes on a sedge meadow, a wetland plant community that builds a deep, peaty soil and is maintained by groundwater discharge. The cooling lake created a 2.75-m (9-ft) hydrostatic head above the remaining wetlands (Figure 3). Seepage through the bottom of the cooling lake has altered the wetland habitat adjacent to the west dike and has helped to dilute the ash effluent as it flows to Rocky Run Creek. Before the generating station was built, groundwater from the adjacent uplands flowed at about 1 ft^3/sec to the sedge meadow (Stephenson and Andrews 1976). Now 1 ft^3/sec seeps into the ashpit drain and mostly into the sedge meadow on the west.

There is evidence that the wetland west of the cooling lake is being altered by a combination of higher and more stable water levels, increased surface water flow and substrate erosion, warmer water temperatures, and perhaps dissolved components (Bedford 1977). Emergent aquatic species and annuals have replaced the previously dominant sedge meadow communities. An equilibrium state has not been reached and effects on vegetation are expected to spread (Bedford 1977). The sedge meadow habitat being replaced has been described as an ideal spawning habitat for northern pike (Priegel and Krohn 1975, McCarraher and Thomas 1972).

Leakage from the ashpit was substantial after it was filled, but has continued only along the west dike where it was not sealed by ash deposits (Stephenson and Andrews 1976). Well-water samples taken in the fall of 1976 indicated the ashpit had some impact on local groundwater chemistry (Andren et al. 1977).

Potential for Acid Rain Damage

Although the pH of rainfall in the Columbia Generating Station vicinity has not been measured, it appears unlikely that acid rainfall will noticeably affect nearby aquatic ecosystems for the following reasons:

1. The Wisconsin River, Rocky Run Creek, and nearby waters are well buffered systems with total alkalinities in the range of 80 to 140 mg/liter $CaCO_3$ and conductivities of 180 to 280 μmhos/cm.

2. Winds are predominately from the west and south (Stearns et al. 1977) and, therefore, power plant emissions should miss most of the nearby aquatic systems located west and south of the plant.

3. The present pH of the Wisconsin River and Rocky Run Creek (7.6 to 8.2) is well within the recommended safe range of pH 6.5 to 9.0 for natural waters and has not changed noticeably since the the plant began operation in 1975.

The effect of added sulfur emissions when Columbia II begins operation should be considered in the future. The contributons, if any, of Columbia plant emissions to acid rainfall over distant waters--such as northern

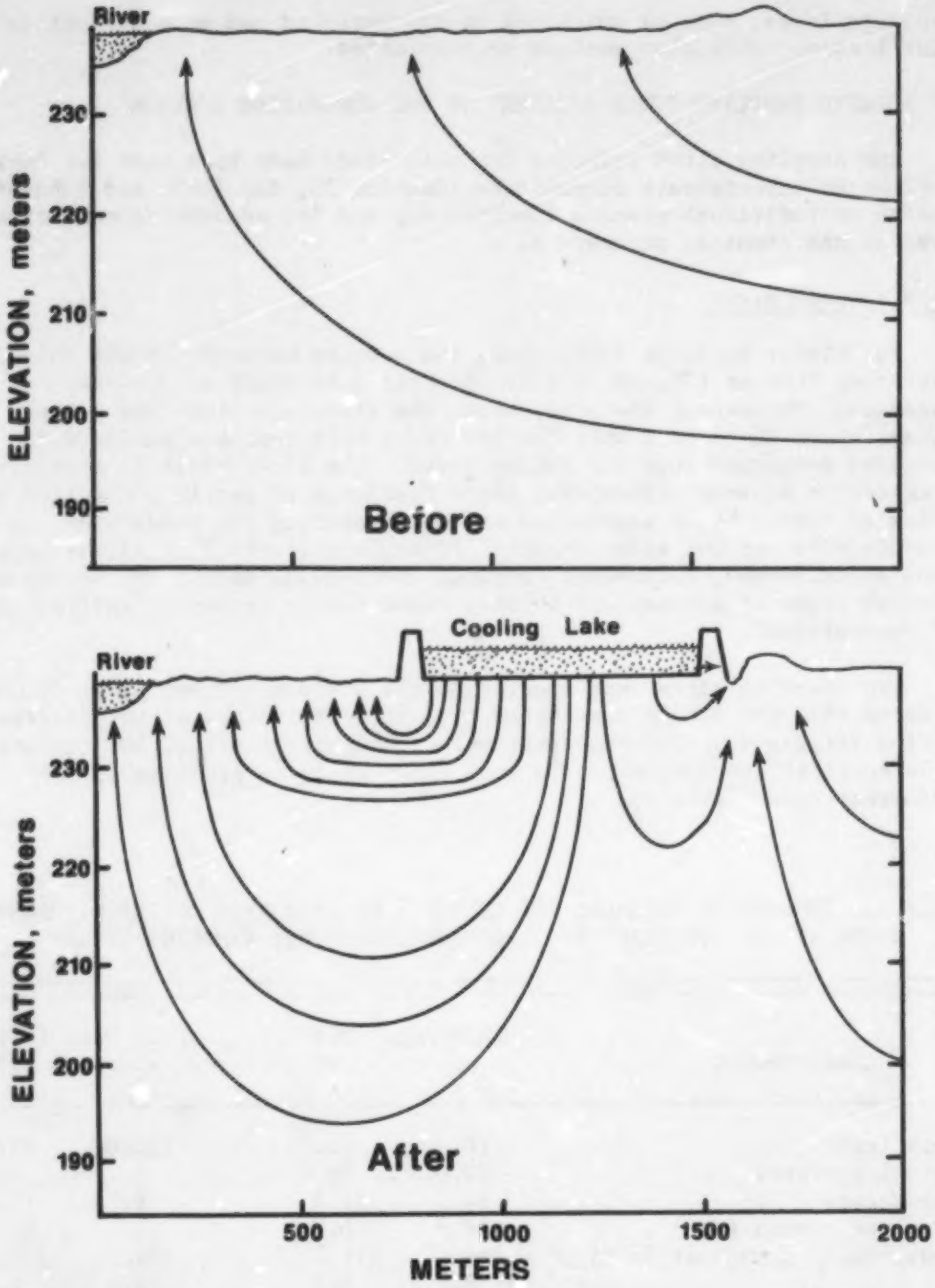

River
230
220
210
200
190
ELEVATION, meters
Before
Cooling Lake
River
230
220
210
200
190
ELEVATION, meters
After
500
1000
1500
2000
METERS

Figure 3. Ground water flows before and after construction of the Columbia cooling lake. Arrows represent integrated flows, 1 m^3/min, normal to the east-west cross section along the length of the cooling lake (from Stephenson and Andrews 1976).

Wisconsin lakes, some of which are poorly buffered and more subject to acidification--will also need to be considered.

THE AQUATIC SAMPLING SITES ADJACENT TO THE GENERATING STATION

The sampling sites selected for this study have been used for field sampling of invertebrate communities (Section 3), for field and laboratory studies on individual species (Section 4), and for associated monitoring of physical and chemical parameters.

The Wisconsin River

The Wisconsin River flows along the western boundary of the Columbia Generating Station (Figure 1) approximately 5 km south of Portage, Wisconsin. Throughout the study area, the Wisconsin River has a sandy bottom, about 25 to 50 m wide (at low flow) with frequent sandbars and with scattered submerged logs and fallen trees. The river basin is characterized by extensive seasonal flooding. Water discharge is partly controlled by a series of dams. Major vegetation types surrounding the study area are floodplain forest and sedge meadow. Floodplain consists of silver maple (*Acer saccharinum*), cottonwood (*Populus deltoides*), willow (*Salix nigra*) and a ground layer of grasses and shrubs. Land use is primarily agricultural and recreational.

Two sampling sites were located on the Wisconsin River about 0.5 km upstream (W1) and 3.0 km downstream (W2) from the intake of the generating station (Figure 1). The two sites were chemically similar, but current and water depth at the upstream site were about twice as great as at the downstream site (Table 1).

TABLE 1. SUMMARY OF PHYSICAL AND CHEMICAL MEASUREMENTS OF THE WISCONSIN RIVER AT THE UPSTREAM (W1) AND DOWNSTREAM (W2) SAMPLING SITES*

Measurement	18 Aug. 1976 W1	18 Aug. 1976 W2	12 Oct. 1976 W1	12 Oct. 1976 W2
Depth (cm)	110.0	56.0	115.0	57.0
Current (cm/sec)	67.0	38.0	--	--
Temperature (°C)	24.0	21.9	14.7	13.0
Dissolved oxygen (ppm)	8.6	8.8	11.4	11.2
Conductivity (mhos at 25°C)	184	211	225	239
pH	8.2	8.3	8.2	8.2
Total alkalinity (ppm)	85.0	91.0	101.0	122.0
Total hardness (ppm)	136.0	128.0	86.0	108.0

* Measurements were made downstream 4 to 5 h earlier than upstream.

Rocky Run Creek

Rocky Run Creek originates in a marsh lake and flows through about 20 km of agricultural lands before reaching the generating station. The creek is spring fed at several points. The substrate is silt with a high organic content. Aquatic vegetation includes water stargrass (*Heteranthera dubia*), pondweed (*Potamogeton* spp.), and coontail (*Ceratophyllum* sp.)

The two most widely separated Rocky Run Creek sites--one upstream from the effluent near Co. Hwy. JV (R1) and one downstream from the effluent near the mouth of the creek (R5)--are separated by a large slough where the ashpit drain discharges into Rocky Run Creek (Figure 1). A main creek channel connects the two sites, but numerous backwaters and small channels are present in the adjacent sedge meadow and flood-plain forest. Flood waters from the Wisconsin River inundate the region in the spring, but water levels gradually drop until only the main creek channel is visible in summer.

Two sampling sites (R1 and R2) in Rocky Run Creek are located upstream from the ash effluent and three sites (R3, R4, R5) are located downstream (Figure 1). The water upstream from the ash effluent is usually higher in alkalinity, hardness, and pH than water at the downstream sites. Table 2 compares stations R1 and R5 and Table 3 presents data for sites R2, R3, and R4. Current speeds and dissolved oxygen are reduced at downstream site R5, but not at the other downstream sites. Conductivity is high near the ash effluent entry and decreases only slightly as it flows to the Wisconsin River (Figure 2). These high conductivities can not be traced into the river itself. Precipitated elements from the ashpit drain form a floc which coats the creek bottom and results in slightly elevated turbidity.

Ashpit Drain

The creek that receives the ashpit effluent originates in wetlands that are part of a mint farm east of the power plant. The creek originally crossed the area now occupied by the cooling lake and entered Duck Creek just above the north knoll (Figure 1). During construction of the Columbia facility, this creek was diverted to run parallel to the east dike of the cooling lake and then turn westward to join the backwaters of Rocky Run Creek. The substrate of the drainage ditch was originally silt with a high organic content. After generating station operation began, some areas (particularly those near site A3) gradually became more sandy as the silt washed away. The artificial drainage ditch banks are steep and grassy for about 0.75 km beyond the railroad tracks. At the end of this diking, sedge meadow and flood-plain forest form the creek banks.

Sedge meadow or drained sedge meadow still border the mint farm creek for several kilometers upstream from site A1. The substrate consists of silt with a high organic content primarily from decomposing sedge.

One sampling station (A1) is located in the mint drain upstream from the ash effluent (Figure 1) and five sites (A2, A3, A4, A5, and A6) follow the effluent gradient to its entry into Rocky Run Creek. Conductivity is

TABLE 2. SUMMARY OF PHYSICAL AND CHEMICAL MEASUREMENTS UPSTREAM AND DOWNSTREAM OF THE ASH EFFLUENT IN ROCKY RUN CREEK. AVERAGES OF MONTHLY SAMPLES (MAY THROUGH OCTOBER), ± 1 S.D.; (RANGE): AND NUMBER OF SAMPLES

	Upstream (R1)		Downstream (R5)	
Measurement	1976	1977	1976	1977
Temperature (°C)	19.7±7.4 (7.3-25.9) 6	17.5±5.9 (8.8-24.0) 6	20.4±6.4 (11.1-26.5) 6	17.2±6.9 (9.1-27.0) 6
Current speed (cm/speed)	25.7±6.2 (15.4-32.1) 5	23.7±2.3 (20.8-26.1) 4	---	< 5
Dissolved oxygen (mg/liter)	12.1±0.8 (10.8-13.3) 6	11.6±0.7 (11.0-12.8) 5	9.3±1.3 (7.7-11.2) 6	8.6±1.7 (5.7-10.0) 5
Conductivity (μmhos/cm) at 25°C	472±14 (454-482)	485±14 (475-512)	437±30 (386-477)	804±280 (528-1188)
Alkalinity phenol (ppm)	9.4±12.6 (0.0-28.5) 7	4.9±9.4 (0.0-23.4) 6	--- --- 3	--- --- 6
Total (ppm)	260.3±21.4 (222.3-283.0) 7	250.1±15.4 (235.0-278.8) 6	225.21±19.6 (200.0-256.5) 7	195.1±25.5 (158.0-228.2) 6
Hardness (ppm)	269.5±2.9 (266.0-273.0) 4	270.9±4.0 (264.8-275.2) 6	244.1±2.6 (240.6-247.0) 4	209.5±21.0 (175.6-234.8) 6
pH	795±0.20 (7.75-8.20) 6	7.87±0.18 (7.65-8.05) 6	7.64±0.21 (7.25-7.06) 6	7.61±0.26 (7.36-7.90) 6
Turbidity (JTU)	---	4.5+1.9 (2.2-7.0) 5.	---	7.0+3.9 (5.0-14.0) 5

outfall (Table 3). Current speeds in the ashpit drain (A2 through A6) are faster than in the mint drain (A1) and alkalinity, hardness, and usually pH are lower. Temperature is usually several degrees higher in the ashpit drain, probably because of leakage from the cooling lake. Some dissolved trace-element concentrations are high in the ashpit, but precipitate after the pH is lowered and before the effluent is discharged to surrounding waters (Table 4). The precipitated elements form the floc that slightly elevates turbidity and coats the bottom of the ashpit drain (Table 3). Although levels of these dissolved trace elements are lower in the ashpit drain than in the ashpit, they are higher than the levels in the mint drain or Wisconsin River. They sometimes exceed the estimated no-effect concentrations which are based on single elements in laboratory bioassays (Water Quality Criteria 1973). There are also some fly-ash particles and perhaps some organic byproducts of coal combustion in the ash effluent.

TABLE 3. SUMMARY OF PHYSICAL AND CHEMICAL MEASUREMENTS UPSTREAM AND DOWNSTREAM FROM THE ASH EFFLUENT IN THE ASHPIT DRAIN AND ROCKY RUN CREEK ON SEPT. 1 AND 9 1977. AVERAGE, ± 1 S.D.; (RANGE); AND NUMBER OF SAMPLES

Measurement	Site							
	A1	A2	A3	A6	S1	R2	R3	R4
Temperature (°C)	18.0±0.0 (18.0) 2	21.5±0.7 (21.0-22.0) 2	21.1±0.1 (21.0-21.2) 2	22.8±1.1 (22.0-23.5) 2	22.3±1.1 (21.0-23.6) 2	20.9±1.3 (20.0-21.8) 2	22.5±0.7 (22.0-23.0) 2	22.2±0.2 (22.0-22.3) 2
Current speed (cm/sec)	+ --- 1	11.4 --- 1	21.4 --- 1	11.1 --- 1	6.1 --- 1	21.6 --- 1	29.7 --- 1	18.2 --- 1
Dissolves oxygen (mg/liter)‡	7.6 --- 1	---	8.6 --- 1	---	---	10.9 --- 1	---	10.3 --- 1
Conductivity (μmhos/cm)	454±20 (439-468) 2	2882±68 (2834-2930) 2	2515±11 (2507-2523) 2	2376±136 (2279-2472) 2	420±12 (429-412) 2	480±11 (472-488) 2	1119±147 (954-1238) 3	1108±67 (1060-1155) 2
Alkalinity phenol (ppm)	0.0 --- 1	0.0 --- 1	0.0 --- 1	0.0 --- 1	0.0 --- 1	4.8 --- 1	0.0 --- 1	0.0 --- 1
Total (ppm)	250.2 --- 1	59.2 --- 1	93.8 --- 1	103.8 --- 1	220.9 --- 1	282.8 --- 1	193.8 --- 1	207.2 --- 1
Hardness (ppm)	244.4 --- 1	120.0 --- 1	130.0 --- 1	145.2 --- 1	197.6 --- 1	272.4 --- 1	204.8 --- 1	210.0 --- 1
pH	7.45 ---	7.25 ---	7.35 ---	7.45 ---	7.25 ---	7.95 ---	7.65 ---	7.65 ---
Turbidity (JTU)	3.8 1	27.0 1	27.0 1	21.0 1	21.0 1	6.2 1	18.0 1	17.0 1

+Strong wind was reversing the current on this date; typical values range from 7 to 9 cm/sec.

‡Oxygen meter malfunctioned. Dissolved oxygen values reported are for 7 Oct. 1977. Temperatures were 9.2, 9.0, 8.0, and 8.0°C, respectively.

TABLE 4. CONCENTRATIONS (PPM) OF SELECTED TRACE ELEMENTS IN DISSOLVED AND SUSPENDED PARTICULATE FRACTIONS OF THE ASHPIT DRAINAGE SYSTEM*

	Wisconsin River	Ashpit discharge**	Mint drain	Ashpit drain	
			(A1)	(A2)	(A4)
Dissolved particulates					
Cr	<0.001-0.001	0.014-0.077	<0.001	0.035-0.065	0.006-0.028
Ba†	<0.050	0.730	<0.075	0.380	---
Al	0.022-0.093	0.1-11.4	0.003-0.165	0.03-4.0	0.045-0.476
Cd	0.0001	0.0021-0.0031	0.0001-0.0002	0.0024-0.0029	0.0001-0.0012
Cu	<0.001-0.002	0.004-0.045	<0.0003-0.002	0.004-0.043	0.002-0.024
Suspended particulates					
Cr	---	134	107	740	1,294
Ba	---	17,240	---	1,350	1,225
Al‡	---	---	---	---	---

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*Dissolved analyses were from monthly samples in November 1976 through April 1977 by Andren et al.(1977). Suspended particulates were measured in the fall of 1975 by Helmke et al. (1976a).

**Before addition of sulfuric acid.

†Helmke et al. (1976b).

‡Aluminum is an important component of the precipitate in the ashpit drain (Andren et al. 1977), but its concentration cannot be measured by neutron activation analysis.

SECTION 2

CONCLUSIONS

Fly ash from the 527-MW coal-fired Columbia Generating Station Unit I (Columbia Co., Wis.) is discharged as a slurry into an adjacent ashpit. Water from the ashpit is pumped to a ditch that joins two streams--the ashpit drain and Rocky Run Creek--before reaching the Wisconsin River. Relatively minor changes in water quality parameters (e.g., alkalinity, hardness, pH, and turbididty), increased amounts of some dissolved trace elements (Cr, Ba, Al, Cd, and Cu), and the precipitation of trace elements (Al, Ba, and Cr) into a floc that coats the bottom of the streams have caused habitat alterations.

Effects of the fly-ash effluent from Columbia I on aquatic invertebrate communities decreased as distance from the generating station increased. The ash effluent concentration changed on a seasonal basis depending on the volume of water pumped from the ashpit and on the amount of dilution from the mint drain creek, sedge meadow flow, Rocky Run Creek, and groundwater discharge. The conductivity of the effluent increased in January 1977 when sodium bicarbonate was first used to increase the efficiency of the electrostatic precipitators. Since then, conductivity measurements have been used to indicate effluent concentration at distances downstream from the generating station.

After Columbia I began operating in 1975, the ashpit drain--the creek that directly receives the ash effluent--became an unsuitable habitat for aquatic invertebrates. There was a 3-month delay before community changes were observed in 1975; some invertebrate taxa thrived late in the fall. However, upstream and downstream comparisons of invertebrate communities in 1977 revealed a lack of organisms colonizing artificial substrates downstream.

Community differences were also observed in Rocky Run Creek--0.5 km downstream from the ash effluent entry--when compared to upstream samples, but only when conductivity was over 1,000 μmhos/cm. Upstream-downstream differences were not detected in Rocky Run Creek when the conductivity was near 800 μmhos/cm. Invertebrates drifting from the upstream station to downstream stations were suddenly exposed to the ash effluent and apparently did not colonize artificial substrates when the effluent concentration was above a threshold level. Invertebrates appeared unaffected 1 km downstream in Rocky Run Creek where pre-operational data were compared to post-operational data.

Effects of the operation of Columbia I were undetectable in the Wisconsin River from 1974 to 1977. Natural variation in seasonal cycles of invertebrate communities in the river were documented; these data will be useful for long-term monitoring of the river.

It was possible to examine the reasons for the differences observed in the field by controlling exposures of individual populations of crustaceans to the ash effluent. Crayfish caged downstream from the ash effluent survived at the same rate as those caged at upstream control sites, but they contained higher levels of five metals (chromium, barium, zinc, selenium, and iron) in their body tissues and had lower metabolic rates. The lowered metabolic rates of exposed crayfish were influenced by one or more of the following: Reduced quantity or quality of food; increased metal concentrations in tissues; or possibly a combination of water quality parameters affected by coal-combustion byproducts.

Concentrations of chromium in potential food sources for invertebrates at the Columbia site increased as much as four-fold in leaf litter and nine-fold in suspended particulates downstream from the ash effluent. Laboratory crayfish exposed to chromium in their food accumulated less than 3% of the amount ingested. However, chromium in food may be an important factor affecting invertebrate populations at the Columbia site because of its high concentration of particulate sources.

Survival of winter-generation *Asellus racovitzai* was similar for exposure to control and ash-effluent water and to control and ash effluent-exposed food. Poor late winter condition of the isopods precluded detection of any sublethal effects. However, young-of-the-year *Gammarus pseudolimnaeus* were more sensitive to the ash effluent than were adults of the species.

Results of these studies can be considered in relation to effluent concentration at any one time and place (Figure 4). Thresholds for field and laboratory responses to the ash effluent were estimated by averaging no-effect and lowest-effect conductivities (Table 5). The threshold for effects fell between 800 and 1,459 μmhos/cm with an average of about 1,100 μmhos/dm. The conductivity gradient downstream of the generating station can be used to predict the extent of the effects.

Conductivity measurements were above the threshold of effects in the ashpit drain before it enters Rocky Run Creek during all of 1977-78 (Figure 5). Exceptions occurred when the generating station was not operating for short periods in the spring and fall of 1977. Conductivity in Rocky Run Creek was lower than in the ashpit drain, usually near the 1,100 μmhos/cm threshold and higher on one occasion. Responses of invertebrate communities were more subtle in the creek than in the ashpit drain and were more difficult to assess. Although Rocky Run Creek is still a suitable habitat for many aquatic invertebrates, evidence of sublethal stress and habitat avoidance exists.

It is hypothesized that the major effect of the operation of Columbia I on aquatic invertebrates is through habitat alteration and in particular, through reduced substrate quality and avoidance of unpreferred habitat. Susceptibility of early life stages to the ash effluent may also be

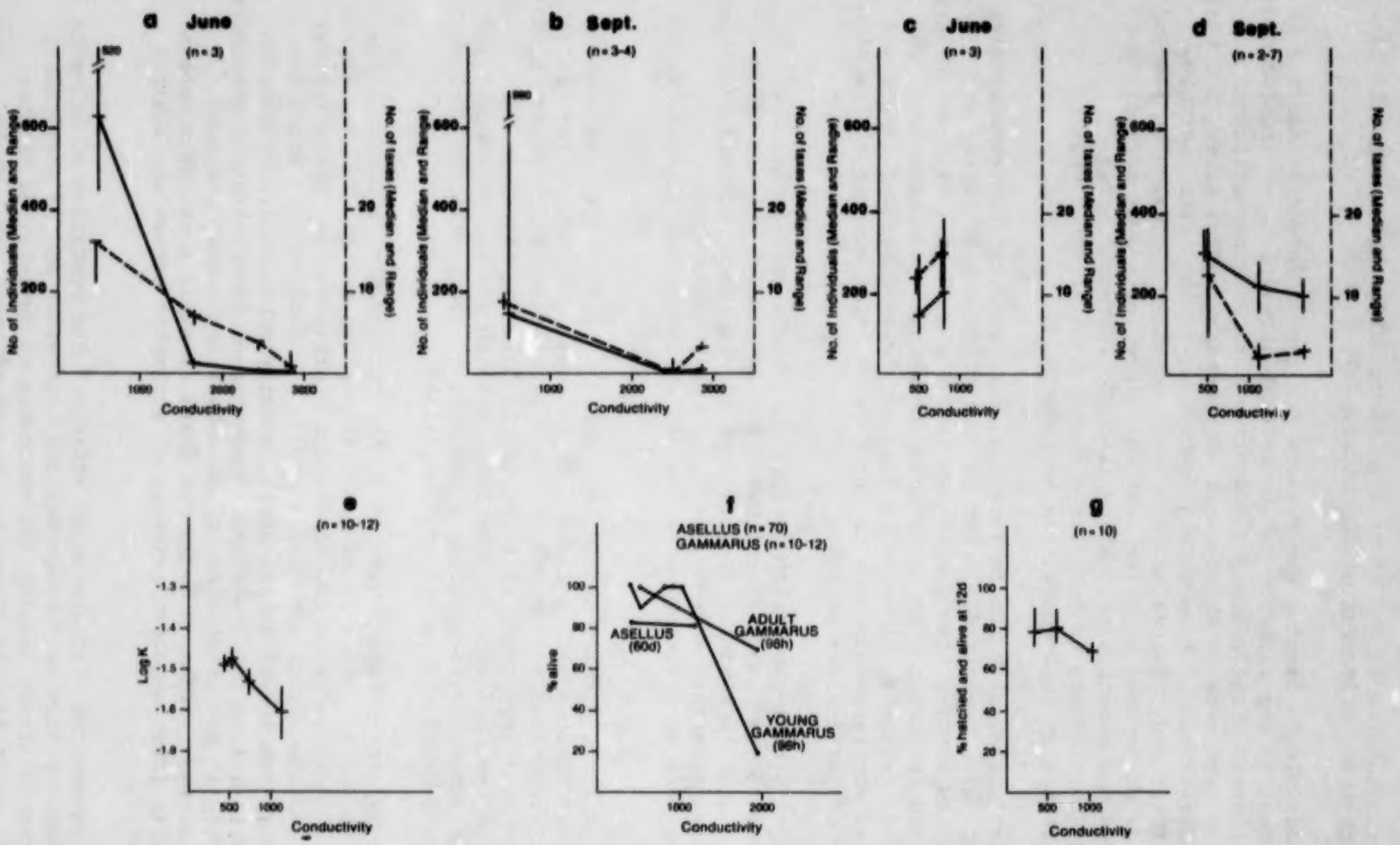

Figure 4. Summary of the effects of the ash effluent in field and laboratory experiments. Ash-effluent concentration is expressed by conductivity (μmhos/cm) of the water. Modified Dendy samplers were placed upstream and downstream from the ash effluent in the ashpit drain (a and b) and Rocky Run Creek (c and d). Crayfish (e) were exposed in the field for 62 days and their metabolic rates were measured in the laboratory ($K = mgO_2\ h^{-1}\ g^{-1}$). *Asellus* and *Gammarus* (f) and northern pike eggs (g) were exposed to ash effluent in the laboratory.

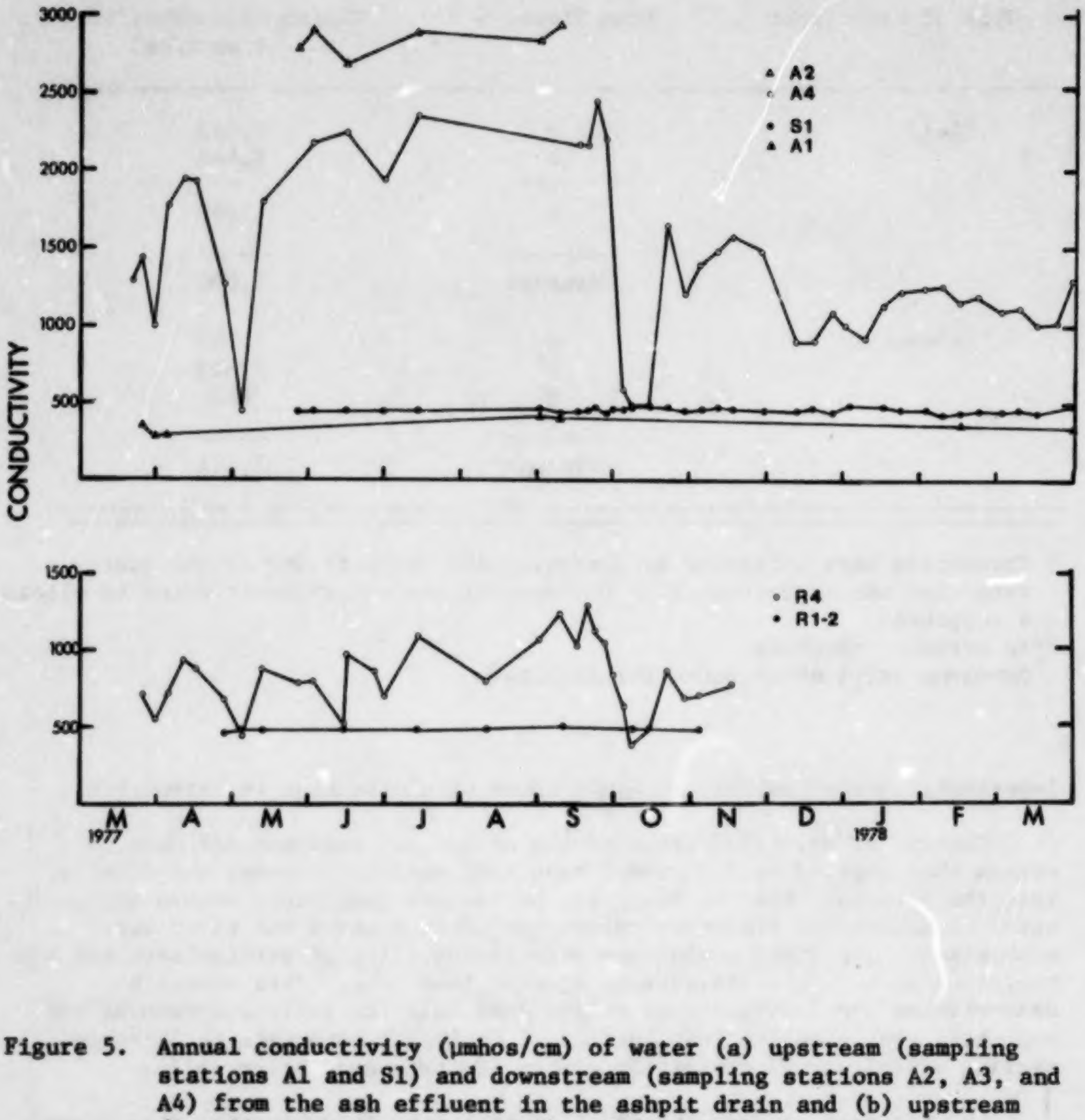

Figure 5. **Annual conductivity (μmhos/cm) of water (a) upstream (sampling stations A1 and S1) and downstream (sampling stations A2, A3, and A4) from the ash effluent in the ashpit drain and (b) upstream (sampling stations R1 and R2) and downstream (sampling stations R3 and R4) in Rocky Run Creek in 1977-78.**

TABLE 5. ESTIMATED THRESHOLDS FOR BIOLOGICAL RESPONSES TO ASH EFFLUENT (FROM FIGURE 4, a through g)*

Type of experiment	From Figure 4	Threshold conductivity (mhos/cm)
Field	a	1,042
	b	1,448
	c**	--
	d	800
	Average	1,096
Laboratory	e	1,148
	f†	1,459
	g	825
	Average	1,144

* Thresholds were estimated by averaging the conductivity of the control water and the conductivity of the most dilute ash effluent water to elicit a response.
**No response observed.
†*Gammarus* only; no response for *Asellus*.

important. Acute toxicity to adult forms of crustaceans is unimportant.

Guthrie et al. (1974) studied the effects of coal-ash effluent on a stream that emptied into a swamp, then into smaller streams, and finally into the Savannah River in Georgia. Mechanisms that would return effluent water to acceptable standards before the water entered the river were emphasized. Important mechanisms were the settling of particulates and the recycling of chemical elements by aquatic food webs. This research demonstrated the importance of entire food webs for pollutant removal and suggested the selective introduction of resistant consumers to increase the cycling efficiency of the biotic system (Guthrie and Cherry 1976).

The drainage system for ash effluent at the Columbia site also flows through small streams and wetland habitat before reaching the Wisconsin River. In contrast to the Georgia study, the Columbia wetland has been valued as a habitat for spawning game fish (Magnuson et al. 1980) and resident and migratory birds (Willard et al. 1977). Inputs from the coal-ash effluent and changes in the characteristics of the wetland vegetation adjacent to the cooling lake (Bedford 1977) threaten habitat quality for wildlife. Our concern with long-term effects of the generating station is focused equally on habitat loss and on the quality of water entering the Wisconsin River. Wetland habitats available for spawning fish populations in this section of the Wisconsin River were inventoried (Magnuson et al. 1980) and it was determined that the generating station site provides at least 30% of the available habitat.

In summary, it was concluded that the 3.6-km long ashpit drain has become an unsuitable habitat for aquatic invertebrates. The habitat quality of a localized area of Rocky Run Creek at least 0.5 km downstream of the ashpit drain entry has also been reduced. These localized effects will increase as more effluent is discharged and effects will be most evident in low-water years when effluent dilution is minimal.

SECTION 3

EFFECTS ON COMMUNITY STRUCTURE OF MACROINVERTEBRATES

INTRODUCTION

The purpose of this chapter is to document the impact of the Columbia Generating Station on aquatic invertebrates in the Wisconsin River, Rocky Run Creek, and the ashpit drain. Of particular interest is the community of invertebrates inhabiting the two streams that receive the ash effluent--the ashpit drain and Rocky Run Creek (Figure 1). Sample sites were selected upstream (A1, R1, and R2) and downstream (A2 through A6, and R3 through R5) from the ash effluent in both streams to observe effects of a dilutional gradient. Effects of the ash effluent were not measureable in the Wisconsin River (W1 and W2), but seasonal cycles of macroinvertebrates as baseline data for long-term change were documented.

Aquatic invertebrates have often been used to indicate environmental quality and change (Wilhm 1975). Analytical techniques provide indices of community diversity from data on numbers and kinds of species in a series of samples. Traditional techniques produce diversity indices for one sample at a time. Multivariate techniques present similarities and differences between many samples simultaneously; therefore, assemblages of many species can be compared in space and time.

Ordination as a multivariate tool was used to compare samples because it gives a graphic representation of complex relationships. Patterns in the data are not readily discernable with the more classical analytical methods. Ordination has been used in macroinvertebrate studies to observe community change along physical gradients such as salinity, temperature, or substrate type (Hughes and Thomas 1971; Erman 1973; Hocutt 1975). It also has been used to demonstrate the ordering of a series of macroinvertebrate stations along a gradient of many pollutional disturbances (Beckett 1978). In this study ordination was used to examine:

1. Similarities between different stations on a single date

2. Changes in the communities at different stations on a seasonal basis

3. Changes in the seasonal cycle of organisms from year to year.

For comparison, more traditional methods of representing community diversity were used: the Shannon-Weaver index (Shannon and Weaver 1949), evenness (Pielow 1966), equitability (Lloyd and Gherlardi 1964), and the number of taxa and individuals.

MATERIALS AND METHODS

The invertebrate community was sampled using artificial substrates so that colinization at different stream sites could be assessed without the complication of variable substrate type. The resulting samples are quantitative but are not measures of actual standing crops. Two types of artificial substrate samplers--basket-type with limestone and a modified Dendy-type--were used to sample the invertebrate community. Both upstream-downstream and before-after operation sampling designs were used (Tables 6 and 7).

Basket-Type Artificial Substrates

Five sampling sites were selected in 1974 for the pre- and post-operational study (Figure 1). Three were downstream from the generating station. The first (A3) was in the ashpit drain about 1.8 km downstream from the confluence of the ash effluent with the mint farm creek, the second (R5) was near the mouth of Rocky Run Creek, and the third and farthest downstream site (W2) was in the Wisconsin River about 0.5 km from the mouth of Rocky Run Creek. Two sampling sites were located upstream from the generating station, one (W1) on the Wisconsin River about 0.4 km upstream from the intake channel and the other (R1) on Rocky Run Creek near Co. Hwy. JV.

Monthly samples were taken after spring floods until freeze-up in 1974 and 1975. Sampling continued at the downstream Wisconsin River site in 1976 and at both Rocky Run Creek stations in 1976 and 1977 (Table 6).

Organisms were collected from basket-type artificial substrate samplers (Mason et al. 1970) which consisted of 20- x 29-cm chicken barbeque baskets (or similar wire replicas) filled with 4.5 kg of limestone gravel (average diameter of 7.6 cm) and suspended from overhanging branches 5 to 10 cm above the substrate. Three samplers were placed at each station. At approximately 1-month intervals, the organisms were removed by shaking the samplers about 12 times inside an aquatic D-frame net (1-mm mesh). In the Wisconsin River samplers, nets of Hydropsychidae larvae often held rocks together; when this occurred, the basket was shaken until the rocks were loose.

All samples were preserved in 70% alcohol in the field. Insects and crustaceans were sorted, identified, and counted in the laboratory. Samples were divided into four subsamples before sorting. If more than 2.5 h was needed to sort the first 25% of a sample, the sample was subsampled. Downstream Rocky Run Creek samples (R5) were never subsampled.

Identification was usually to the generic or sometimes family level; specific identifications were made where possible. The smallest instars of the Hydropsychidae and Corixidae families could not be identified to genus; separate categories were created for them. Pupae were keyed to family.

TABLE 6. MONTHLY SAMPLING SCHEDULE FOR BASKET-TYPE ARTIFICIAL SUBSTRATES*

Location	Month	Day			
		1974	1975	1976*	1977
Wisconsin River upstream (W1) and downstream (W2)					
	May	10	28	24	--
	June	10	26	22	--
	July	14	23	21	--
	August	15	21	17	--
	September	13	19	15	--
	October	11	17	12	--
Rocky Run Creek upstream (R1) and downstream (R5)					
	May	10	28	24	31
	June	10	26	22	28
	July	14	23	21	27
	August	15	21	17	25
	September	13	19	15	23
	October	11	17	12	21
Ashpit drain (A3)					
	May	--	28	--	--
	June	14	26	--	--
	July	15	23	--	--
	August	13	21	--	--
	September	10	19	--	--
	October	12	17	--	--
	November	19	--	--	--

*Dates shown are midpoints between sampler placement and removal. 1974 was the pre-operational year. Samplers were placed after the peak of the spring flooding each year.

**Downstream only for the Wisconsin River.

Modified Dendy Samplers

The invertebrate community was sampled upstream and downstream of the ash effluent in June and September of 1977 (Table 7). Artificial substrates were modified from the multiple-plate Dendy sampler (Hester and Dendy 1962). Each of these modified Dendy samplers was made from a single Tuffy brand mesh ball held between two 8- x 8-cm masonite plates by an eye bolt and wing nut. Samplers were held in place with floats and weights. Because modified Dendy samplers could be placed anywhere in the stream, it was possible to randomly sample the creeks at sites closer to the ash effluent. Placement of the basket-type artificial substrates had required overhanging branches. The smaller size of the Dendy samplers made it possible to process more replicates. Three samplers were randomly placed at sites A1, A2, A3, R2, and R3 in June; four samplers at A1, A2, A3, A5, A6, and S1 in September; and eight samplers at R2, R3, and R4 in September (Figure 1). After a 1-week exposure, the samplers were removed and placed in freezer containers with 70% alcohol. The 1-week exposure time was selected to minimize the buildup of detritus around the sampler. Longer exposure times were not practical because of this buildup. At the laboratory, samples were concentrated through a 0.07-mm net and organisms were identified and counted.

TABLE 7. SAMPLING SCHEDULE FOR MODIFIED DENDY SAMPLERS AND NUMBER OF SAMPLERS PLACED UPSTREAM AND DOWNSTREAM OF THE ASH EFFLUENT FOR 1-WEEK COLONIZATIONS

Location	Station	Date removed 6 June 1977	 9 Sept. 1977
Mint drain	A1	3	4
Ashpit drain	A2	3	4
	A3	3	4
	A4	0	4
Sedge meadow flow	S1	0	4
Rocky Run Creek			
Upstream	R2	3	8
Downstream	R3	3	8
	R4	0	8

Although eight samplers were placed at stations R2, R3, and R4 in September, several samplers were missing when collections were made. Seven samplers remained at R2, two at R3, and four at R4. For some analyses, samples from the two downstream sites, R3 and R4, were combined to provide a total of six downstream replicates. Samplers were also missing at stations A5 and A6 on the same date. These losses were due to sudden, extremely high ashpit drain flow or were removed by boaters.

Supporting Physical-Chemical Data

Water temperatures were taken whenever samplers were placed or removed. Dissolved oxygen (YSI Model 54A oxygen meter or winkler; American Public Health Association 1971), conductivity (YSI Model 33), pH (Fisher Accumet Model 150 or Hach Phenol Red kit), alkalinity and hardness (American Public Health Association 1971) and current speed (Drogue, Ocean Equipment Model 451, or Neyrpic Midget) were measured irregularly in 1974 and 1975 and whenever samplers were placed or removed in 1976 and 1977. Turbidity was measured in 1977 (Hach Model 2100A). All conductivity readings were adjusted to 25°C.

Methods of Analysis

Polar Ordination--

Many dominant taxa and numerous seasonal or spatial changes make comparisons among stations and years difficult to describe. Ordination techniques simplify these comparisions by accounting for all species and stations simultaneously. Ordination plots can illustrate temporal differences as trajectories connecting samples in time (Bartell et al. 1977). They can also show spatial changes in species composition along ecological gradients or reveal spatial or temporal patterns by clumping similar samples. Samples that represent important changes or that are notably different are often evident as endpoints of the axes or as isolated points. In interpreting ordinations, it is useful to remember that the first axis usually illustrates the greatest (and possibly most important) differences between samples. Successive axes usually account for less variation.

Analyses were performed using three data transformations (numeric, relative abundance, and presence-absence) to assess the influence of taxa with varying numerical importance. Numeric (raw) data were averaged from the three basket-type samplers in each collection. Numeric data used in ordinations emphasized dominant taxa the most, as it also did in relative abundance form, but the latter put samples on an equal numerical basis. The presence-absence form weighted the presence or absence of each species equally, regardless of numerical abundance. Three axes were constructed for each ordination, but the third axis seldom revealed useful information.

Polar ordination was chosen for this study because it involves less ecological distortion and because the results tend to be more ecologically interpretable than results from other mathematically more sophisticated methods of ordination (Gauch and Whittaker 1972, Beals 1973). In polar rdination, distances are calculated between all pairs of samples based on their compositional similarity using the Bray-Curtis Dissimilarity

Coefficient.[1] Axis endpoints were selected with the variance/regression method (Beals et al. unpublished).

Other community measures--

Other indices of species structure in communities are based on calculations from single samples. Some of these methods were applied to this data:

Number of taxa

Number of individuals

The Shannon function (Shannon and Weaver 1949, Wilhm 1970), based on information theory: $\overline{H} = -\sum_{i=1}^{s} pi \log_2 pi$; where s = total number of taxa in a sample and pi is the proportion of individuals in the i[th] taxon. This index was recommended by the U.S. Environmental Protection Agency (U.S. EPA) for purposes of establishing uniformity between different studies (Weber 1973). $\overline{H}$ is influenced by the number of taxa (s) and by the evenness (e) with which individuals are apportioned between the species; it is relatively independent of total sample size (N) (Odum 1971).

Evenness, measured by the function $e = \frac{\overline{H}}{\log s}$ (Pielow 1966) and by the recommended U.S. EPA method, the equitability ratio $E = \frac{s'}{s}$; where s' is the hypothetical number of species based on MacArthur's model (Lloyd and Gherlardi 1964).

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[1] Distances are calculated between all pairs of samples based on their compositional similarity using the Bray-Curtis dissimilarity coefficient[a]:

$$^{a}\ d(Xi, Xj) = \frac{\sum_{k=1}^{n} X_{ik} - X_{jk}}{\sum_{k=1}^{n} X_{ik} + \sum_{k=1}^{n} X_{jk}}$$

where d = distance between sample pairs and n = number of species.

Statistical Analyses--

Statistical tests were used where appropriate to compare invertebrate distributions. The position of samples on ordination axes and the other measures of community diversity listed above were compared between years using a Friedman two-way analysis of variance by ranks (Siegel 1956). Samples collected upstream and downstream from the ash effluent in 1977 were analyzed with the Mann-Whitney U test (Siegel 1956).

RESULTS

Pre- and Post-operational Sampling of the Ashpit Drain and Rocky Run Creek

Samples of invertebrate communities from the basket-type artificial substrates enabled us to compare a pre-operational year (1974) with post-operational years in the ashpit drain (1975) and Rocky Run Creek (1975, 1976, and 1977). In the ashpit drain, considerable changes in the community colonizing the substrates were observed in the post-operational year, 1975. In Rocky Run Creek, community variations during 1975, 1976, and 1977 were subtle and impossible to relate to generating station operation.

Ashpit Drain--

When numbers of organisms were important with numeric data in an ordination and when dominant taxa were emphasized with relative abundance data, the first axis distinctly separated August, September, and October of the post-operational year from all other months (Figures 6a and b). Reductions in number of taxa (s) and individuals (N) occurred in these 3 months (Figure 7). Only the September 1975 sample separated because a number of taxa were absent; this is seen on the first axis of the presence-absence ordination (Figure 6c). A reduction in number of taxa was also apparent in July of the pre-operational year. This sample separated on the second axis of the presence-absence ordination. However, the reduction was temporary; larger numbers of taxa appeared during the rest of the year (Figure 7).

Examples of taxa that were common in 1974 and early 1975, but were absent or greatly reduced during the last 3 months sampled in 1975 (August, September, and October) are *Stenacron interpunctatum*, *Hydropsyche* sp., *Cheumatopsyche* sp., juvenile Hydropsychidae and Chironomidae (Figure 8). Other taxa such as Coenagrionidae, Lepidoptera, and Simuliidae appeared in the fall of 1974, but did not reappear in similar numbers in the fall of 1975. New taxa did not replace these losses or reductions. A few taxa were unchanged in number (for example, *Hyalella azteca,* sp., and *Asellus racovitzai*).

The Shannon index H for pre- and post-operational ashpit drain samples revealed lower diversity in August, September, and October of the first post-operational year (Figure 7). In this case, H gave a useful representation of large community changes in response to the ash effluent. H could be used in combination with the numbers of taxa, numbers of individuals, and the numeric data to draw conclusions similar to those

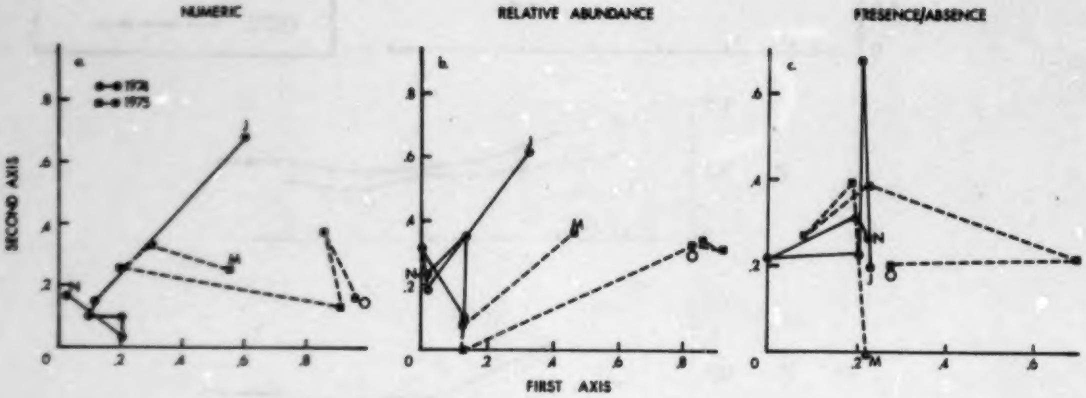

Figure 6. Polar ordination of invertebrate samples from basket-type artificial substrates in the ash pit drain (sampling station A3) using numeric data, relative abundance data, and presence-absence data. Monthly samples were collected from June (J) through November (N) in the pre-operational year (1974) and from May (M) through October (O) in the post-operational year (1975).

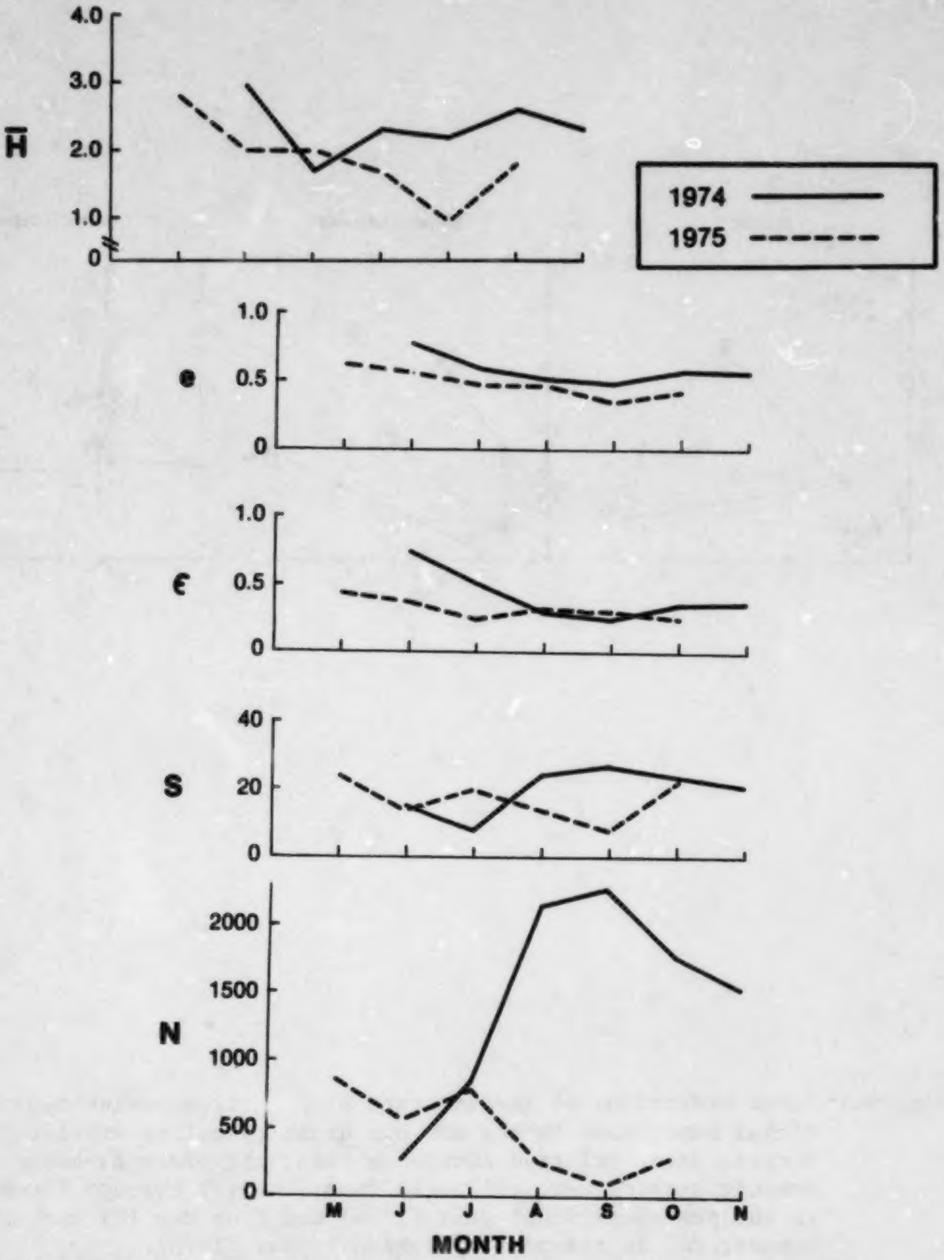

Figure 7. Measures of community diversity in invertebrate samples from basket-type artificial substrates in the ash pit drain (sampling station A3). The Shannon-Weaver index ($\overline{H}$), eveness (e), equitability (E), number of species (S), and number of individuals (N) were calculated for monthly samples from June (J) through November (N) in the pre-operational year (1974) and from May (M) through October (O) in the post-operational year (1975).

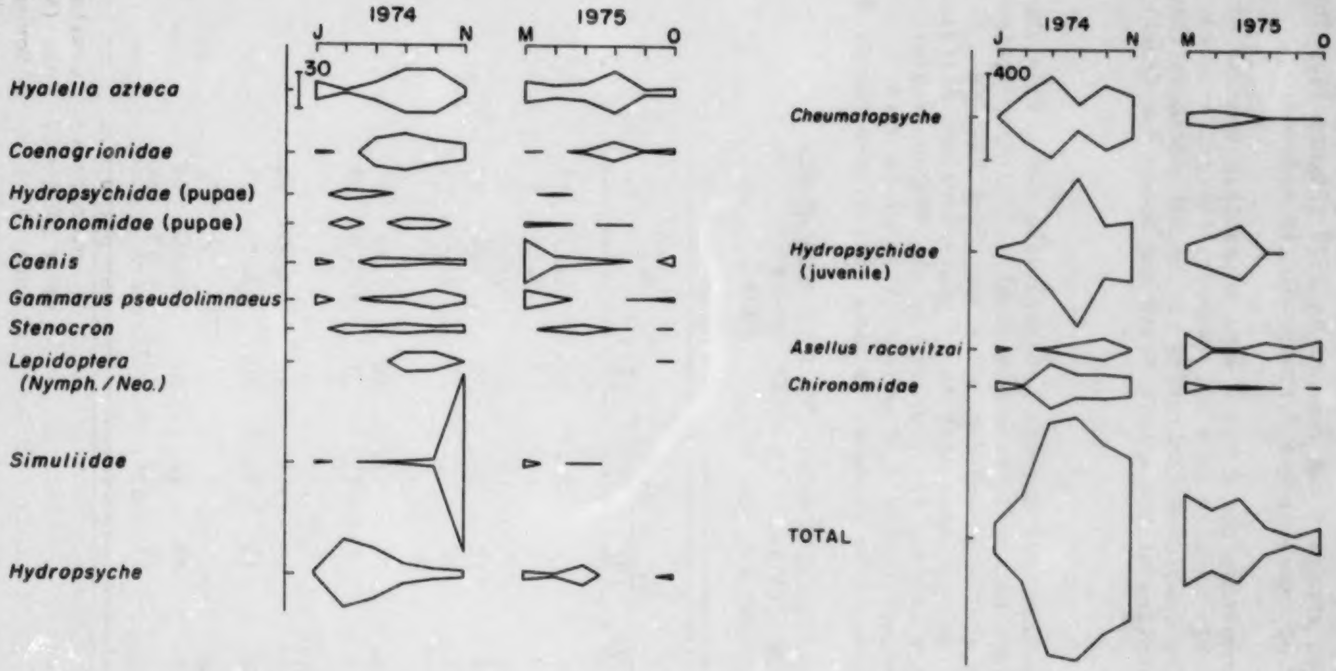

Figure 8. Seasonal abundance of dominant invertebrate taxa (numeric data) in basket-type artificial substrates in the ash pit drain (sampling station A3). Data were collected from June (J) through November (N) in the pre-operational year (1974) and from May (M) through October (O) in the post-operational year (1975).

resulting from the use of ordinations and numeric data. Evenness (e) and equitability (E) were not particularly useful for showing pollution responses; the degree of apportionment for individuals among the taxa was similar in 1974 and 1975 even though H was reduced in 1975 (Figure 7). This occurred because the number of taxa (s) was correspondingly reduced.

Statistical analyses comparing the 2 years were impossible because of small numbers of paired monthly samples ($n = 5$). However, the differences observed formed the basis for continued monitoring of the ash effluent (See "Upstream and Downstream Sampling of the Ashpit Drain and Rocky Run Creek").

Rocky Run Creek--

Sets of six monthly samples (May through October) were taken from the basket-type substrate samplers during 1 pre-operational (1974) and 3 post-operational (1975-77) years at the downstream Rocky Run Creek station (R5). Statistical analyses demonstrated that the 4 years were not different from each other in the number of taxa (S) present, in the Shannon-Weaver diversity index (H), in evenness (e) or equitability (E), or in axis positions from relative abundance or presence-absence ordinations (Table 8).

TABLE 8. STATISTICAL DIFFERENCES AMONG THE FOUR SETS OF SIX MONTHLY SAMPLES (1974-77) IN ROCKY RUN CREEK DOWNSTREAM FROM THE ASH EFFLUENT (R5)[†]

Parameter	Sum of ranks[‡]				r^2	
	1974	1975	1976	1977		
S	15	12	20.5	12.5	4.55	ns
N	19	7	19	15	9.60	*
H	15	17	14	14	0.60	ns
e	14	19	12	15	2.60	ns
E	17	18	11	14	3.00	ns
Numeric ordination						
Axis 1	8.5	21	14.5	16	9.90	*
Axis 2	18	6	15	21	12.05	**
Relative abundance ordination						
Axis 1	13	12	15	20	3.80	ns
Axis 2	20	14	16	10	5.85	ns
Presence/absence ordination						
Axis 1	10	16	13	21	6.60	ns
Axis 2	20.5	16	12	11.5	6.15	ns

* $p < 0.05$.
**$p < 0.01$.

[†] Friedman two-way analysis of variance by ranks.
[‡] Ranked from lowest to highest values.

Significant differences were observed in comparing the total number of organisms or in comparing the axes from numeric ordination. From the sum of ranks for total number of organisms, it is clear that the numbers were lowest in 1975. The sum of ranks for axes from numeric ordination was highest for 1975 on the first axis and lowest for 1975 on the second axis. One might argue that generating station operation affected numbers of invertebrates, but not taxonomic proportions in 1975, and that recovery occurred before the 1976 samples were collected. However, there is data for only a single pre-operational year, and there is no evidence that the drop in numbers is beyond the range of natural variation. We believe that this amount of fluctuation would occur in the absence of the generating station.

Since differences between years were based only on numerical data and similarities were reflected in all other community measures tested, it is concluded that generating station operation had no observable effect at this sampling station.

A second approach to the analysis of Rocky Run Creek samples treated the pre-operational year as a control by subtracting data for each month from the respective months in the post-operational years. This was done for the ordinations by directly measuring distances between pre- and post-operational samples for each month from graphs of the first and second axes. The result was one ranking procedure for each ordination.

All parameters for the post-operatonal years varied randomly from the pre-operational year except for the Shannon-Weaver index (H) and the relative abundance ordination (Table 9). The index H was most similar to control in 1975, as shown by the low sum of ranks. The seasonal changes in H were nearly identical in 1974 and 1975 (Figure 9). H sometimes varied from 1974 in 1976 and 1977, but the extent or pattern of the fluctuations did not indicate that the operation of the generating station was a cause.

Differences from the control year on the relative abundance ordination axes were pronounced in 1977 (Table 9); the sum of ranks is larger than in 1975 or 1976. At this point, it is useful to look at the ordination axes before attempting an explanation.

For each of the three ordinations (numeric, relative abundance, and presence-absence), trajectories connecting the six monthly samples for each year were separated for viewing (Figure 10). The first axis in the numerical and relative abundance ordinations spread the samples on a seasonal basis, with fall samples occurring near the origin. Fall peaks of *Pelocoris femoratus* and *Hyalella azteca* populations (Figure 11) contributed to this. The seasonal cycles in different years appear quite similar. The midsummer samples in 1977 (July and August) show the greatest differences from other years; these samples are at the endpoints of the second axis for numerical data (Figure 10d) and are compressed nearer the origin (as compared with midsummer 1974-76 samples) for relative abundance data (Figure 10h). Changes in taxonomic composition in the three post-operational years, as compared to the control year, were statistically significant in the relative abundance ordination. The differences, however, are subtle and cannot be attributed to generating station operation. The most obvious

TABLE 9. STATISTICAL DIFFERENCES AMONG THREE SETS OF SIX POST-OPERATIONAL SAMPLES (1976-1977) IN ROCKY RUN CREEK DOWNSTREAM FROM THE ASH EFFLUENT (R5) AS COMPARED TO THE CORRESPONDING SET OF SIX CONTROL OR PRE-OPERATIONAL SAMPLES (1974)†

Parameter	Sum of ranks ‡			r^2		
	1975	1976	1977			
S	11.5	13.5	11	2.04	ns	
N	15	11	10	3.82	ns	
H	7	17	12	9.94	*	
e	15	9	12	3.74	ns	
E	9	15	12	4.50	ns	
Ordinations						
Numeric	15	10	11	3.82	ns	
Relative abundance	8.5	9.5	18	10.70	**	$p < 0.001$
Presence/absence	13	8	15	5.52	ns	

*$p < 0.05$.
**$p < 0.001$.
†Friedman two-way analysis of variance by ranks.
‡Ranked from least to greatest difference from 1974.
§Based on distances from plots of Axis 1 vs. Axis 2.

differences in 1977 are midsummer population increases in *Asellus racovitzai*, *Dubiraphia* sp., and *Caenis* sp., and decreases in *Berosus* sp. (larvae) (Figure 11).

Community diversity, as illustrated by H, e, and E, declined in the fall of each year (Figure 9), a pattern that can be attributed to the dominance of *Pelcoris femoratus* and *Hyalella azteca*. Although the diversity functions demonstrate a certain predictability in community structure through the sampling season at this location, the ordinations were useful for: 1) showing when yearly seasonal abundance cycles were similar because of taxonomic composition and 2) encouraging us to examine the midsummer samples for subtle changes in community structure in 1977.

Data were also collected at the upstream Rocky Run station (R1) to compare the magnitude of year-to-year change between upstream and downstream stations. These data are being stored and may be used if changes caused by the generating station are eventually observed downstream. The invertebrate community at the upstream station (Figure 12) was quite different from the downstream station because of habitat differences and especially because of faster current upstream.

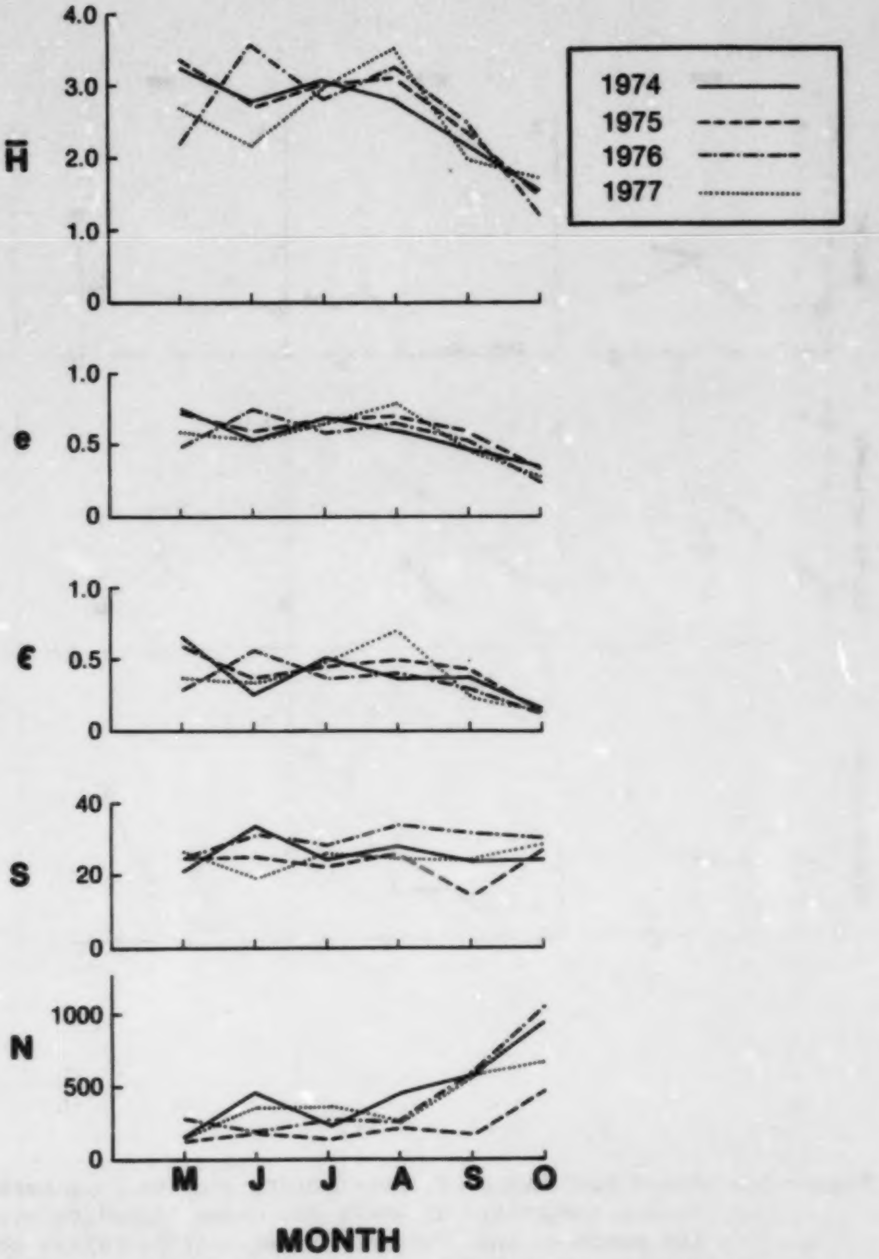

Figure 9. Measures of community diversity in invertebrate samples from basket-type artificial substrates in Rocky Run Creek (sampling station R5) near the mouth of the Wisconsin River. The Shannon-Weaver index ($\overline{H}$), evenness (e), equitability (E), number of species (S), and number of individuals (N) were calculated for each of six monthly samples from May (M) to October (O) in pre-operational year (1974) and 3 post-operational years (1975-77).

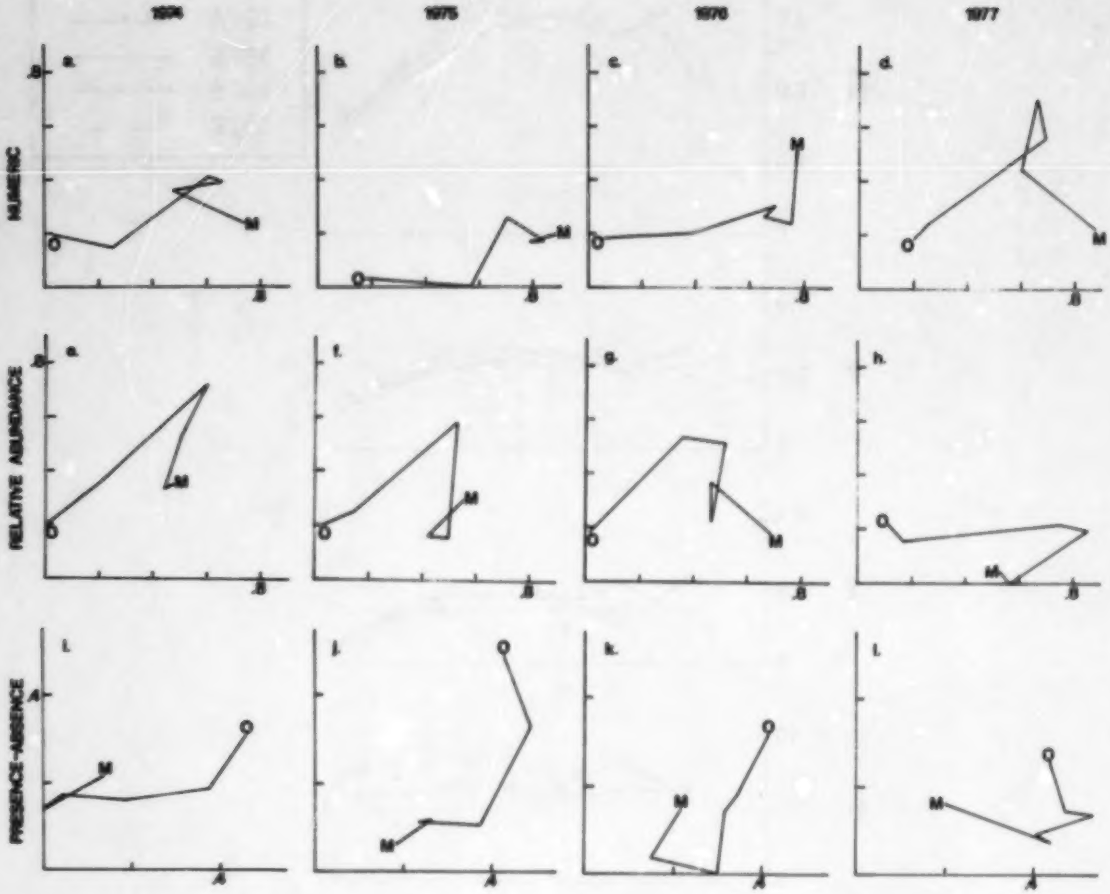

Figure 10. Polar ordination of invertebrate samples from basket-type artificial substrates in Rocky Run Creek (sampling station R5) near the mouth of the Wisconsin River. Trajectories connect six monthly samples from May (M) through October (O) for 1 pre-operational year (1974) and 3 post-operational years (1975-77). All four trajectories for numeric data (a through d) are based on one ordination and are separated for clarity. The same is true for all four trajectories for relative abundance data (e through h) and for presence/absence data (i through l).

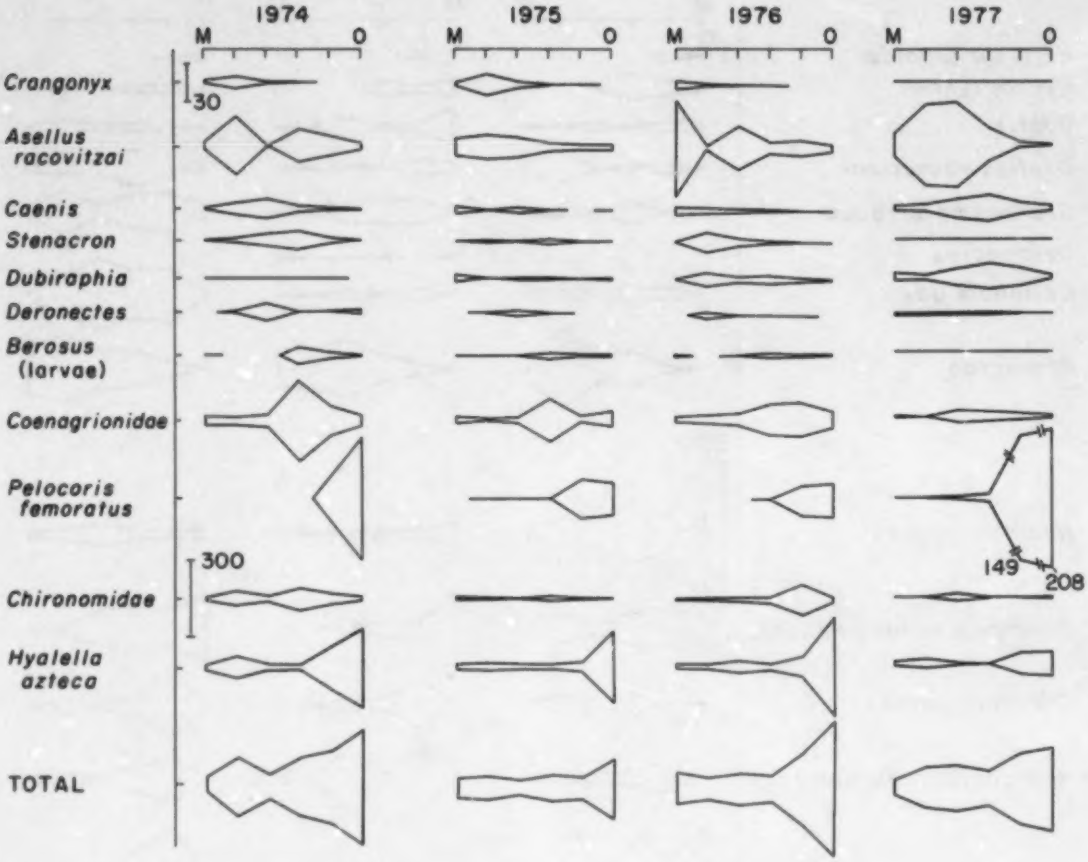

Figure 11. Seasonal abundance of dominant invertebrate taxa (numeric data) in basket-type artificial substrates at the downstream station in Rocky Run Creek (sampling station R5). Data were gathered from May (M) through October (O) in 1 pre-operational year (1974) and 3 post-operational years (1975-77).

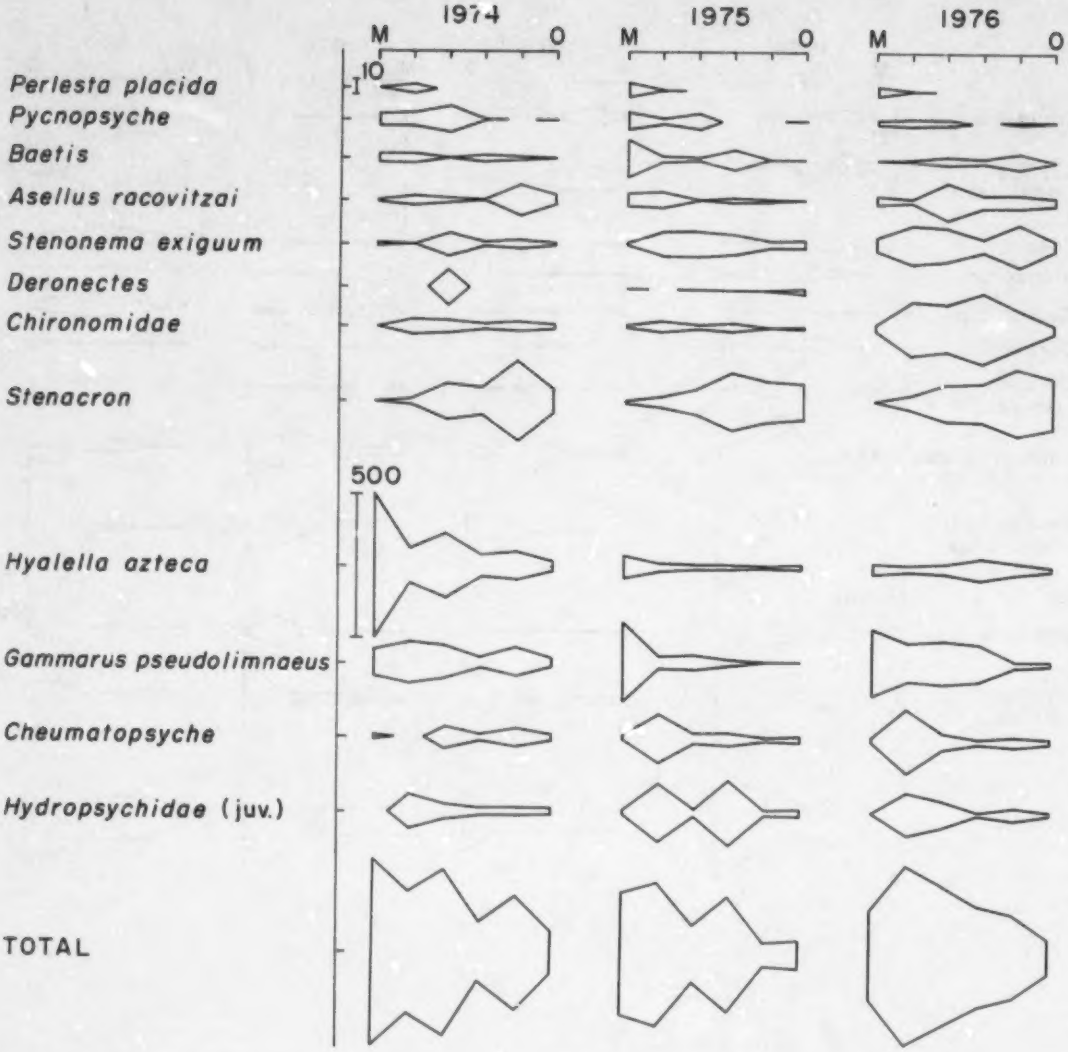

Figure 12. Seasonal abundance of dominant invertebrate taxa (numeric data) in basket-type artificial substrates at the upstream station in Rocky Run Creek (sampling station R1). Data were gathered from May (M) through October (O) in 1974, 1975, and 1976.

Upstream and Downstream Sampling of the Ashpit Drain and Rocky Run Creek

The modified Dendy substrates were used to compare invertebrate communities closer to the ash effluent entry than was possible with basket samplers. The data were collected in the third post-operational year 1977 which was the first year conductivity could be used to indicate ash effluent concentration.

Numbers of taxa and individuals per modified Dendy sampler were extremely low in the ashpit drain, as compared to the upstream mint creek in June and September 1977 (Figure 13). Conductivity was over 2,000 mhos/cm in the ashpit drain, indicating high effluent concentration. During the June sampling, water was being drawn out of the cooling lake into the sedge meadow, and hence into Rocky Run Creek. This diluted the ash effluent to less than 800 mhos/cm downstream in Rocky Run Creek and the invertebrate community colonizing the artifical substrates was not affected. In September, the dilution from the sedge meadow was significantly reduced and the conductivity in downstream Rocky Run Creek was greater than 1,000 µmhos/cm. The number of individuals colonizing the substrates downstream as compared to upstream declined.

Polar ordinations of the September Rocky Run data were used to interpret the nature of community differences between upstream and downstream samples. The differences in numbers of individuals in the upstream and downstream samples was apparent on the first axis of ordination using numeric data (Figure 14d). The percentage ordination demonstrated a tendency for upstream and downstream samples to separate on the second axis, but it can be concluded that the dominant species are similar at both locations (Figure 14b). An ordinaton of presence/absence data resulted in the separation of upstream and downstream samples on the first axis (Figure 14c). The second axis separated the two downstream samples occurring closest to the ash effluent. The series of ordinations indicated that total numbers of individuals were different downstream from the ash effluent, that community structure was similar as far as dominant species are concerned, but that total taxonomic composition differed.

We selected the 13 most abundant of 29 taxa[2] from the above samples and hypothesized that differences in number would be statistically lower downstream using the Mann-Whitney test on each taxon ($p < 0.05$). The hypothesis held true for 10 of the 13 taxa, as well as for the total numbers of individuals (Table 10).

There are three upstream sources of organisms that can potentially colonize Rocky Run Creek downstream from the ash effluent: 1) the ashpit drain, 2) upstream Rocky Run Creek, and 3) the flow from the sedge meadow. The ashpit drain can be excluded because of the near absence of organisms. Ordinations of the upstream and downstream Rocky Run Creek samples and the sedge-meadow samples demonstrate that community structure in the sedge-

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[2] Excluded taxa were represented by less than four individuals and were present in only a few of the samples.

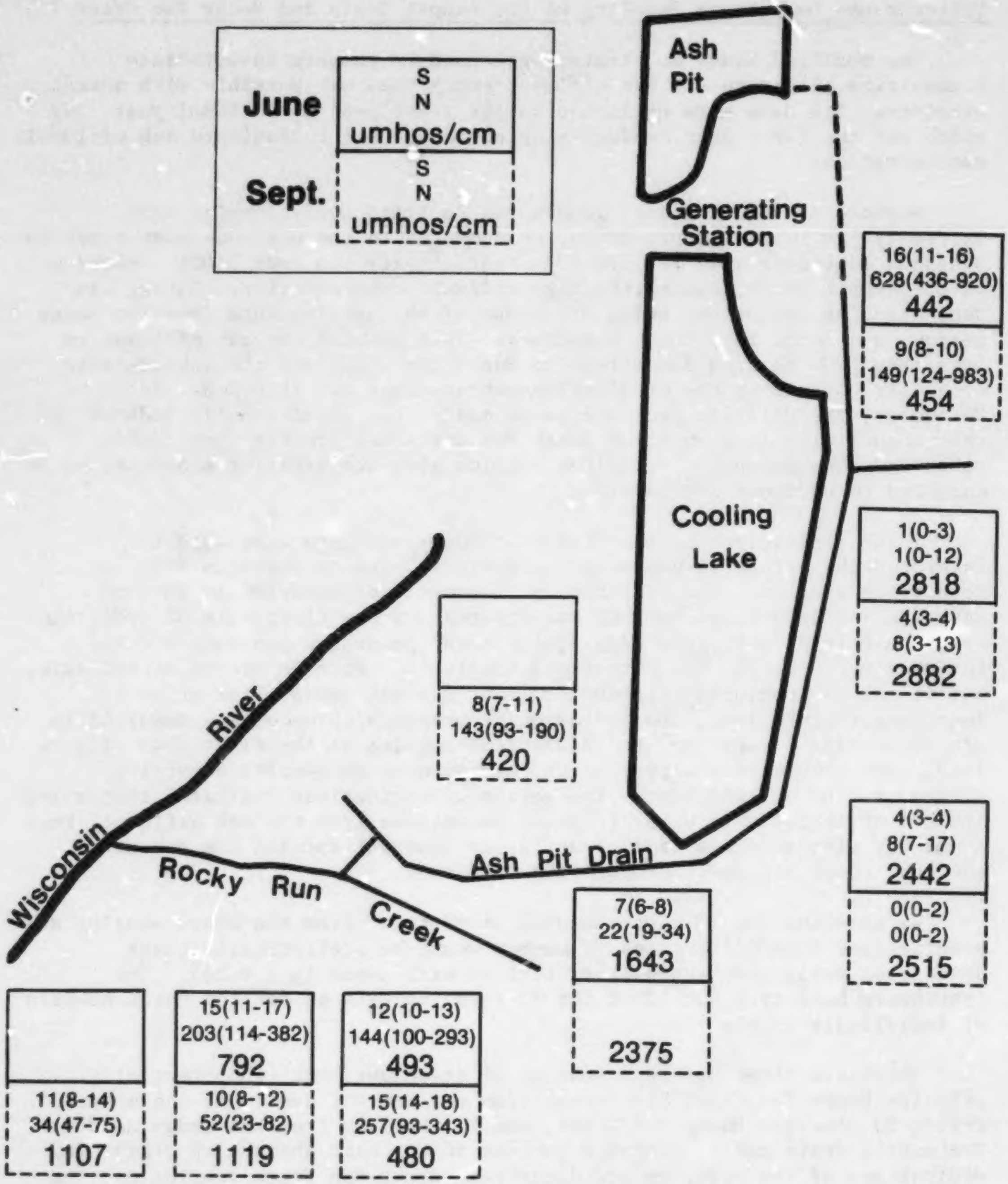
June
S
N
umhos/cm
Sept.
S
N
umhos/cm
Ash
Pit
Generating
Station
16(11-16)
628(436-920)
442
9(8-10)
149(124-983)
454
Cooling
Lake
1(0-3)
1(0-12)
2818
4(3-4)
8(3-13)
2882
8(7-11)
143(93-190)
420
Wisconsin
River
Rocky
Run
Creek
Ash
Pit
Drain
4(3-4)
8(7-17)
2442
0(0-2)
0(0-2)
2515
7(6-8)
22(19-34)
1643
2375
11(8-14)
34(47-75)
1107
15(11-17)
203(114-382)
792
10(8-12)
52(23-82)
1119
12(10-13)
144(100-293)
493
15(14-18)
257(93-343)
480

Figure 13. Number of invertebrate taxa (S) and number of individuals (N) colonizing modified Dendy samplers upstream and downstream from the ash effluent. Medians (and ranges) are reported for June and September 1977. Conductivity (μmhos/cm) is included as an indication of effluent concentration.

TABLE 10. SIGNIFICANCE OF DIFFERENCE IN THE NUMBERS OF ORGANISMS IN ROCKY RUN CREEK, ABOVE AND BELOW THE ASHPIT DRAIN.

Crustacea	*Gammarus pseudolimnaeus*	*
	Hyalella	*
	Asellus racovitzai	n.s.
Ephemeroptera	*Baetis* spp.	*
	Stenacron interpunctatum	*
	Stenonema exiguum	*
Trichoptera	Hydroptilidae	n.s.
	Hydroptilidae pupae	n.s.
	Cheumatopsyche sp.	*
	Hydropsyche sp.	*
	Hydropsychidae (small instars)	*
Diptera	Chironomidae	*
	Simuliidae	*
Total number of individuals		*

*Numbers significantly lower below the ashpit drain at $p < 0.05$, Mann-Whitney U, $n_1 = 6$ (R3 and R4 combined), $n_2 = 7$.

meadow flow (relative abundance and presence/absence data) was different from all Rocky Run Creek samples on the first axis (Figure 15b and 15c). Numbers of dominant sedge-meadow taxa were intermediate between the two Rocky Run Creek stations; differences in community structure did not appear until the second axis of the numeric ordination (Figure 15a). It was concluded that the sedge-meadow flow makes a relatively small contribution to the downstream Rocky Run Creek invertebrate community.

Sampling of the Wisconsin River

Seventy-seven different invertebrate taxa, representing 12 orders, were collected from the Wisconsin River. Dominant taxa were three genera of the net-spinning caddisflies (Hydropsychidae--*Hydropsyche, Cheumatopsyche,* and *Potomya* , midges (Chironomidae), and the mayfly genera *Baetis, Baetisca, Caenis, Isonychia, Stenonema,* and *Heptagenia*. Many of the remaining taxa were present only sporadically in the basket samplers; 21 taxa were caught on only one or two dates at the upstream station, and 19 appeared only once or twice downstream. Other taxa occured at low lvels throughout most of the season, never reaching more than 12% of the total community on any one date.

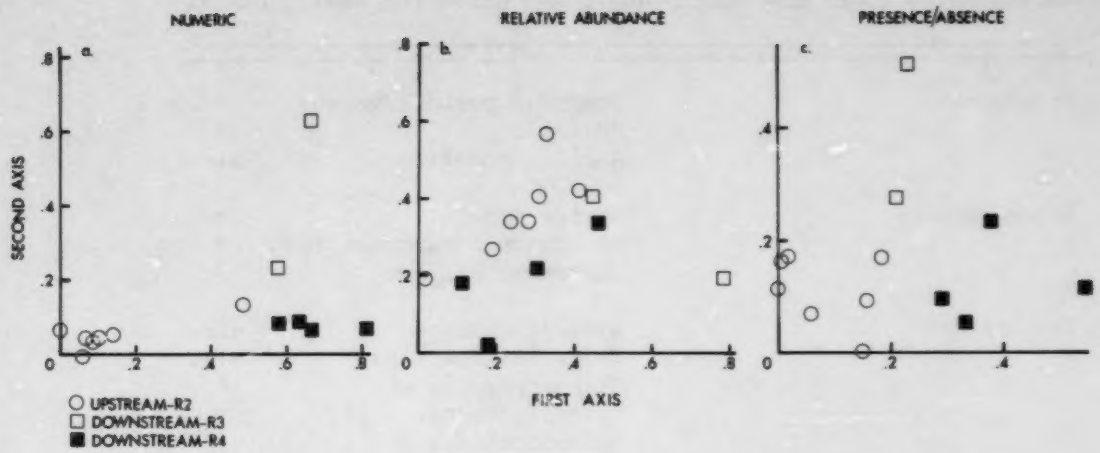

Figure 14. Polar ordination of invertebrate samples from modified Dendy substrates in Rocky Run Creek upstream (sampling station R2) and downstream (sampling stations R3 and R4) from the ash effluent in September 1977.

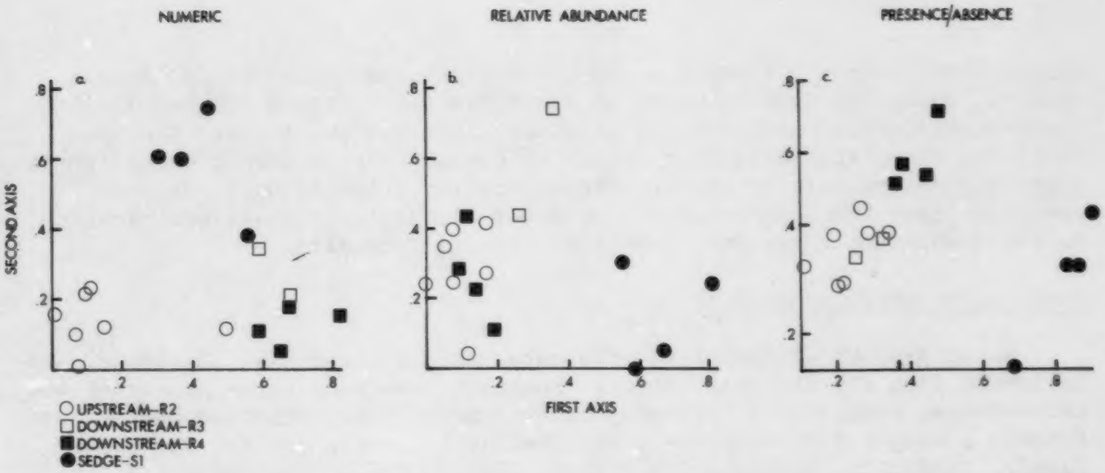

Figure 15. Polar ordination of invertebrate samples from modified Dendy substrates in Rocky Run Creek (sampling stations R2, R3, and R4) and the sedge-meadow flow (station S1) in September 1977.

Wisconsin River data were analyzed for two different purposes: 1) to compare locations downstream from the generating stations for 3 years (one pre-operational and two post-operational) and 2) to consider the variability in the seasonal cycles of river invertebrate communities.

There were no significant differences between the 3 years sampled downstream from the generating station when the numbers of individuals, the Shannon-Weaver index, or most of the ordination axes were considered (Table 11). Number of taxa was significantly different; the sum of ranks was low in 1974 as compared to 1975 and 1976. Evenness (e) and equitability (E) also demonstrated significant differences between years, with a high sum of ranks in 1974. This would be expected because diversity was not different but the number of taxa was lower in 1974. In examining the raw data, it was obvious that while the smaller number of taxa (s) occurred throughout 1974, the actual numerical difference was small (Figure 16).

TABLE 11. STATISTICAL DIFFERENCES AMONG THE THREE SETS OF SIX MONTHLY SAMPLES (1974-76) FROM THE DOWNSTREAM WISCONSIN RIVER STATION (W2)†

Parameter	Sum of ranks‡			r^2	
	1974	1975	1976		
S	7	15	14	7.90	*
N	9	12	15	4.50	ns
H	14	10	12	2.80	ns
e	17.5	9.5	9	9.18	*
E	18	9	9	10.62	**
Numeric ordination					
Axis 1	14	15	7	7.90	*
Axis 2	12	15	9	4.50	ns
Relative abundance ordination					
Axis 1	14	10	12	2.80	ns
Axis 2	14	12.5	9.5	3.20	ns
Presence/absence ordination					
Axis 1	15	11	10	3.80	ns
Axis 2	10	13	13	2.46	ns

*$p < 0.05$.
**$p < 0.001$.
†Friedman two-way analysis of variance by ranks.
‡Ranked from lowest to highest values.

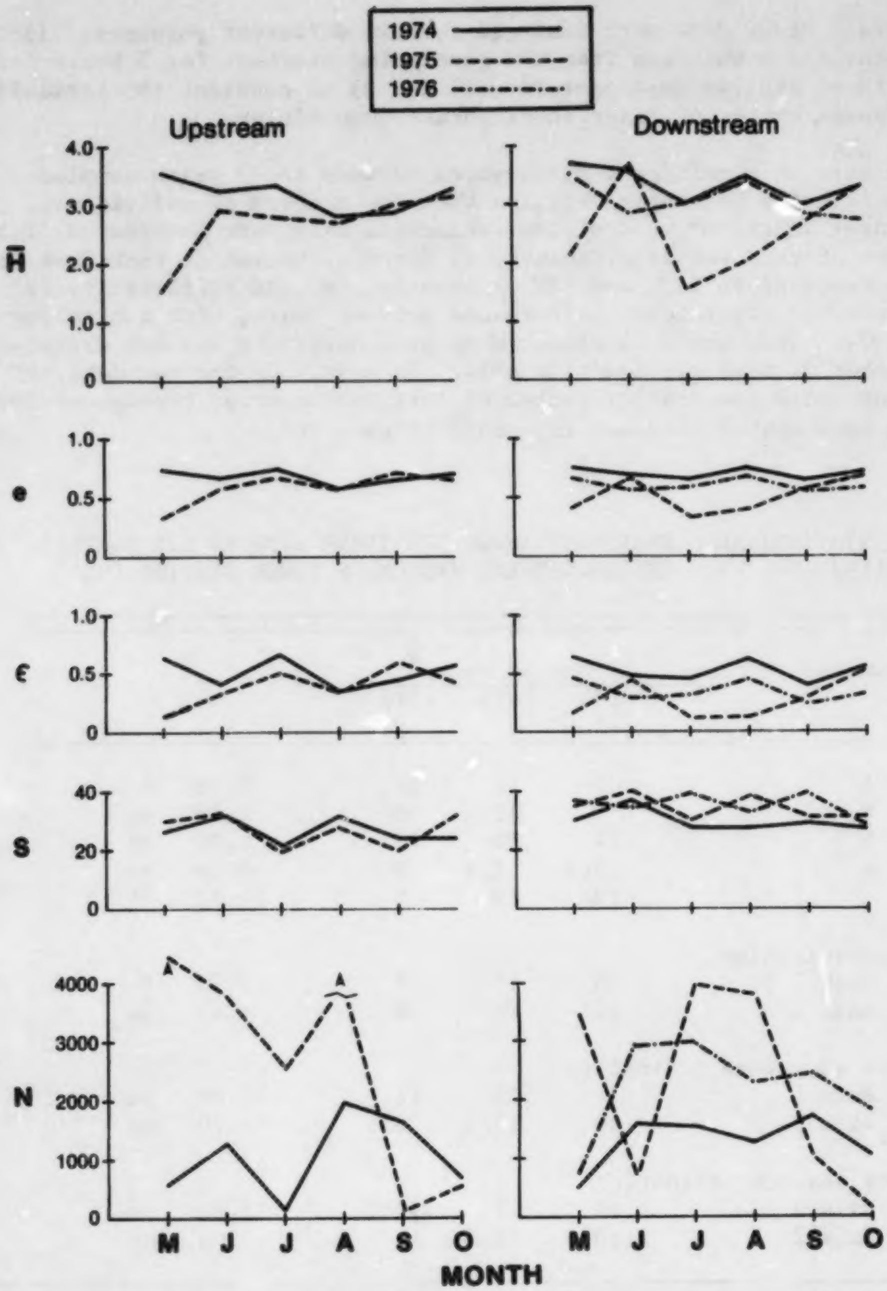

Figure 16. Measures of community diversity in invertebrate samples from basket-type artificial substrates at sites upstream (W1) and downstream (W2) from the Columbia Generating Station. The Shannon-Weaver Index ($\overline{H}$), evenness (e), equitability (E), number of individuals (N) were calculated for monthly samples from May (M) through October (O).

Analyses of sample positions on the first axis of the numeric ordination revealed significant differences between the 3 years (Table 11). None of the other ordination axes demonstrated differences between years. Community structure, as indicated by relative abundance of taxa or by presence and absence of taxa, was therefore similar in 1974, 1975, and 1976.

In summary, none of the above analyses indicated effects of generating station operation. Sampling was limited in only one pre-operational year (1974). Assuming that the variation observed would have occurred in the absence of the generating station, the natural variations in seasonal cycles of river community will now be considered.

All 30 samples (five sets of six monthly samples) were included in each of the three ordinations (numeric, relative abundance, and presence-absence). The five time-series trajectories were difficult to view on one graph, so they have been separated for interpretation (Figure 17). Seasonal change usually accounted for the greatest variation, as shown by the spread of each series of dates on the first axis. Seasonal change often continued to be important on the second axis, resulting in somewhat similar trajectories in different years (for example, Figure 17h and j) or places (for example, Figure 17f and h). The seasonal pattern appeared in about one-half of the plots of the first and second axes as a loop, with the communities at the end of the sampling season (fall) coming back near those at the beginning (spring) (for example, Figure 17a, i, and k).

Numeric Data--

Ordinations using numeric data had samples with the greatest number of organisms nearer the origin of Axis 1 and samples with the smallest number located at the opposite end (Figure 17 a through e). Numerical differences between samples were of continued importance on Axis 2 where spring samples with extremely high numbers of juvenile Hydropsychidae (Figure 18) were located at the end of the axis (Figure 17b and d).

Upstream and downstream seasonal cycles were similar in 1974 (Figure 17a and c), with seasonal loops showing similarities between spring and fall samples. The pattern (Figure 17b and d) was different in 1975 when the total numbers of organisms varied widely during the sampling season (Figure 18). The pattern downstream in 1976 (Figure 17e) was much like the 1974 pattern (Figure 17 c), but the loop was closer to the origin of Axis 1; there was a greater total number of organisms (N) throughout 1976 as compared to 1974 (Figure 18).

Relative Abundance Data--

Transforming data to relativized form placed samples on an equal numerical basis. Ordinations of upstream and downstream samples were again similar in 1974 (Figure 17f and h); however, the 1975 samples not only differed from 1974 but at midsummer they were at opposite ends of the first axis (Figure 17g and i). The taxa with the greatest number of total organisms in July and August 1975 were different upstream and downstream

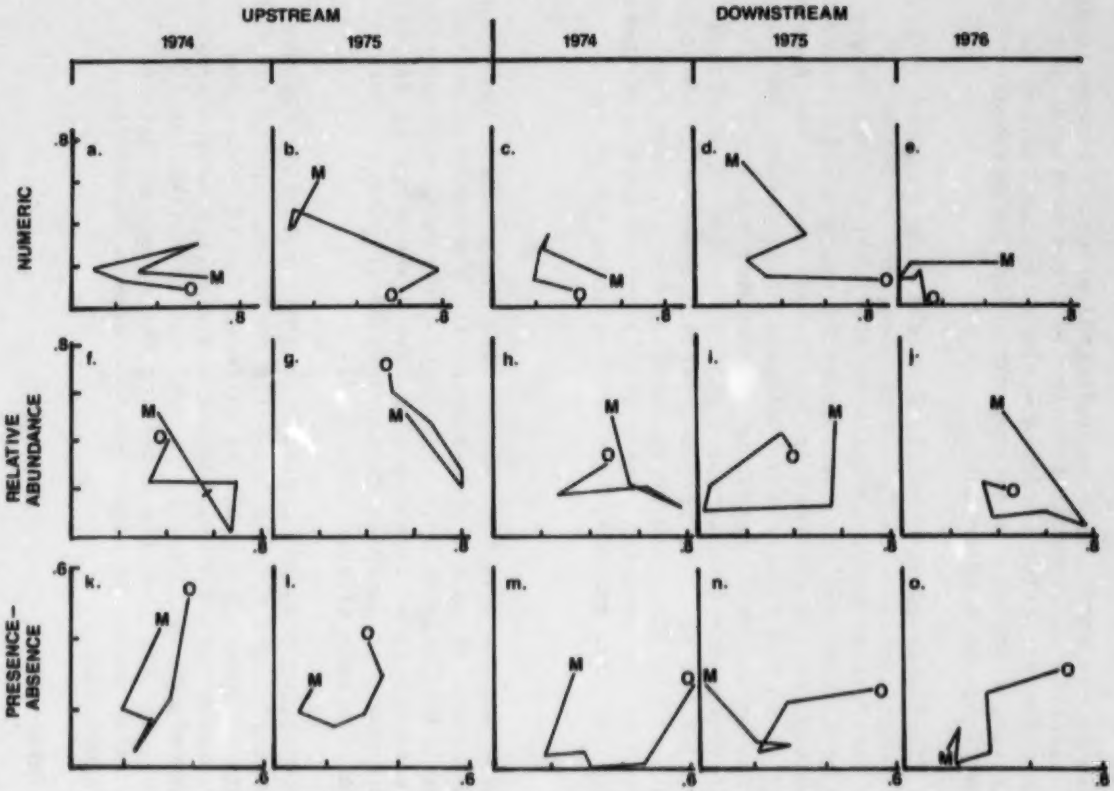

Figure 17. Polar ordination of invertebrate samples from basket-type artificial substrates at sites upstream (W1) and downstream (W2) from the Columbia Generating Station. Trajectories connect six monthly samples from May (M) through October (O) for each location and year. All five trajectories for numeric data (a through e) are based on one ordination and are separated for clarity. The same is true for all five trajectories for relative abundance data (f through j) and for presence-absence data (k through o).

(Figure 18). Downstream, the Chironomidae comprised 70 to 75% of the samples. Upstream, Chironomidae contributed less than 5% and a combination of juvenile Hydropsychidae (small instars), *Hydropsyche, Baetis, Isonychia,* and *Cheumatopsyche* accounted for 80 to 90% of the total. The upstream midsummer community was more similar to the 1974 community by having several dominant taxa. The downstream 1976 samples (Figure 17j) formed a loop similar to that of 1974 (Figure 17h), indicating that the taxonomic succession was similar in both years.

Presence/Absence Data--

When rare taxa had equal weight to abundant taxa in the ordinations, community differences upstream and downstream became apparent. The large number of taxa (77), however, made the differences difficult to interpret. In general, the upstream samples were separated less on Axis 1 than on Axis 2 (Figure 17k and l) and the reverse occurred for downstream samples (Figure 17m, n, and o). There was a number of taxa, characteristic of areas with slow current speeds, that were found primarily at the downstream station where slower current speeds are typical. These included *Stenacron interpunctatum, Tricorythodes* sp. and *Gomphidae*. Their presence indicated a spatial difference in river communities that became apparent in the ordinations only when the less abundant taxa were given equal importance.

DISCUSSION

Ashpit Drain and Rocky Run Creek

The ashpit drain has become an unsuitable habitat for aquatic invertebrates since Columbia I began operating. There was a 3-month delay before community changes were observed in 1975 and some invertebrate taxa thrived late in the fall. However, upstream and downstream comparisons of invertebrate communities in 1977 revealed an impressive lack of organisms colonizing artificial substrates downstream. Conductivity could not be used as a measure of effluent concentration until 1977. However, the effluent probably became more concentrated as fly ash buildup increased in the ash basin.

Community differences were also observed in an upstream-downstream comparison in Rocky Run Creek when the downstream conductivity was over 1,000 μmhos/cm but not when the conductivity was about 800 μmhos/cm. Invertebrates drifting from upstream station R2 to downstream stations R3 and R4 would have experienced a sudden exposure to the ash effluent and apparently did not colonize artificial substrates when the effluent concentration was above a threshold level. Also, invertebrates appeared unaffected when compared to pre-operational data on two occasions when the conductivity near the mouth of Rocky Run Creek was >1,000 μmhos/cm (Table 12). The taxa near the creek mouth were gradually exposed to the 1,000-μmhos/cm effluent, as shown by conductivity measurements through the season. Perhaps the taxa present during the low current flows at this station were more tolerant to this pollutional stress.

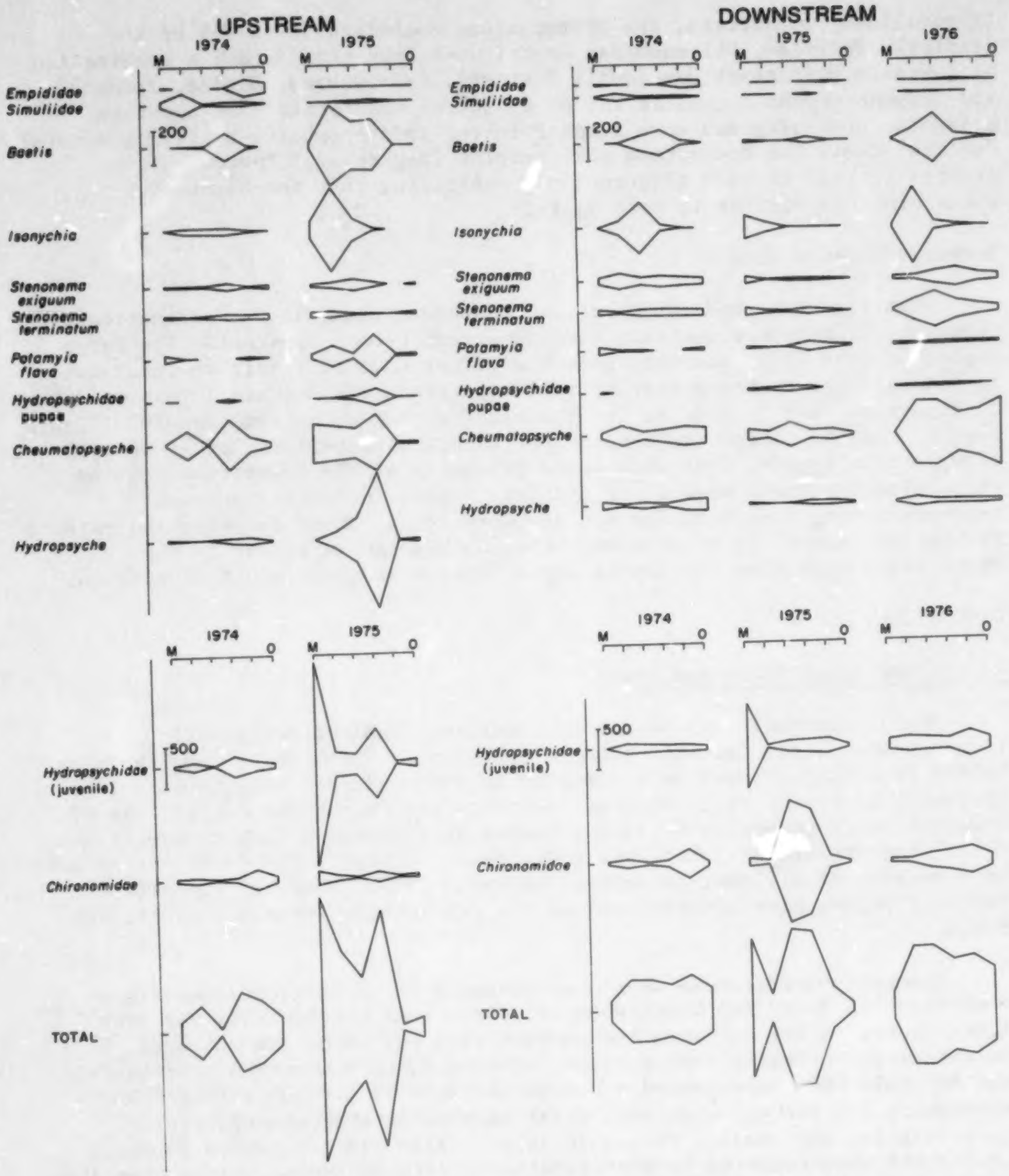

Figure 18. Seasonal abundance of dominant invertebrate taxa (numeric data) in basket-type artificial substrates at the upstream station in the Wisconsin River (W1) from May (M) through October (O) of 1974 and 1975 and the downstream station in the Wisconsin River (W2) from May (M) through October of 1974, 1975, and 1976.

TABLE 12. MONTHLY CONDUCTIVITY AT DOWNSTREAM ROCKY RUN CREEK STATION R5 IN 1977

Month	Conductivity (μmhos/cm)
May	528
June	1,086
July	798
August	1,118
September	530
October	693

It is concluded that the effects of ash effluent decreased in severity as distance from the generating station increased (Table 13). On the basis of field observations, it is hypothesized that thresholds for ash effluent toxicity and/or habitat avoidance exist between 800 and 1,000 μmhos/cm. This is discussed in more detail along with the results of laboratory experiments in Section 2 (Conclusions).

Wisconsin River

None of the community parameters tested for the Wisconsin River samples indicated generating station effects on aquatic invertebrate communities. The effects of the operation of Columbia I were not as yet measurable and research was therefore focused on smaller streams closer to the generating station. Seasonal cycles of invertebrate communities in the river were documented and the data will be of use for long-term monitoring of the river. The collection of data in only 1 pre-operational year (1974) in all streams studied has been and will continue to be a limitation.

Few studies have examined long-term temporal variation in macroinvertebrate communities. Ward (1975) found very little change over 29 years in a relatively undisturbed mountain stream. Richardson (1928) documented more drastic community changes over 12 years in midwestern streams affected by pollution. McConnville (1972) reported consistent seasonal trends during a 2-year period on the Mississippi River.

Other studies have documented spatial variation among widely separated stations within both a stream and single riffle (Needham and Usinger 1956). McConville (1972) found few community differences in a 5-mile section of the Mississippi River. Beckett (1978) used polar ordination to demonstrate faunal homogeneity in a series of 14 stations on a southwestern Ohio river system during the June high water period. The same 14 stations, however, were ordered along a gradient of pollutional disturbances during low flow in August and September when pollutant concentrations were

TABLE 13. RESULTS OF MACROINVERTEBRATE COMMUNITY STUDY

Location	Distance downstream from ashpit (km)	Years sampled	Detectable differences ?
Ashpit drain	3	1974-1975	Yes - before & after
Ashpit drain	1,3,5	1977	Yes - upstream & downstream
Rocky Run Creek	5.5	1977	Yes - upstream & downstream
Rocky Run Creek	6	1974-1975-1976-1977	No - before & after
Wisconsin River	7	1974-1975-1976	No - before & after
			No - upstream & downstream

maximized.

Similar seasonal cycles of aquatic invertebrates were observed in this study at two stations 3.5 km apart in the Wisconsin River in 1974. The time-series trajectories created by the first two axes were very similar for ordinations of numeric and relative abundance data. The 1975 seasonal pattern was quite different from that of 1974 and the relative abundance ordination revealed differences between upstream and downstream samples in midsummer. The 1976 seasonal trajectory was similar to that of 1974. The presence-absence ordination was more difficult to interpret, but showed spatial differences within the river by spreading upstream samples on the second axis and downstream samples on the first axis.

Seasonal change was reflected on the first and/or second axes in the ordinations regardless of the data transformation, but the comparison of several transformations was still useful. For example, use of the numeric data isolated samples with high or low total numbers of organisms. Ordination of relative abundance data helped to show specific patterns in seasonal cycles by eliminating the effect of total sample size without removing the importance of dominant taxa. The presence/absence data made it possible to assess the importance of less abundant taxa by giving all taxa equal weight. The use of several transformations can be applied to pollution studies using ordination. For example, samples with similar diversity but with different dominant taxa or with similar diversity but different numbers can be separated. More ecological information is often revealed than if traditional diversity indices are used alone.

The initial spring placement of our samplers into the Wisconsin River was governed by the timing of receding floods (Figure 19). Samplers were placed after the peak spring floods when danger of damage or loss was reduced. Hence, our year-to-year comparisons of invertebrate community structure were based around water level rather than temperature, photoperiod, etc. One might argue that the differences in the invertebrate community observed in 1975 were due simply to the later onset of the sampling program. However, the 1976 samplers were placed at about the same calendar time as in 1975, but the seasonal cycle was more similar to that of 1974. The year with the most unusual water level was 1975; there were several fall floods. The fall water levels in 1974 were higher than those of 1976, but mean daily river depths varied little in either year. This could help explain the small differences in invertebrate communities upstream between 1974 and 1975, but not the larger differences downstream. A sand bar formed in front of the downstream station in 1975. The offshore portion of the bar was exposed by early July and by early August it was continuous with the shore. The altered current flow caused more siltation at the downstream station and some taxa typical of this habitat were more numerous (e.g. *Stenacron, Tricorythodes,* Gomphidae). The sand bar was covered with water again in 1976 which would explain the greater similarity of 1974 and 1976 trajectories from the downstream site. Other sources of variation in this study include natural influences, man-induced perturbations, and sampling error, although none of the variations observed wre attributed to man-made causes. The study was not designed to assess variability in the river as a whole by randomly selecting many stations, but rather to look at the predictability of patterns at only two locations. A study encompassing both points of view would be valuable.

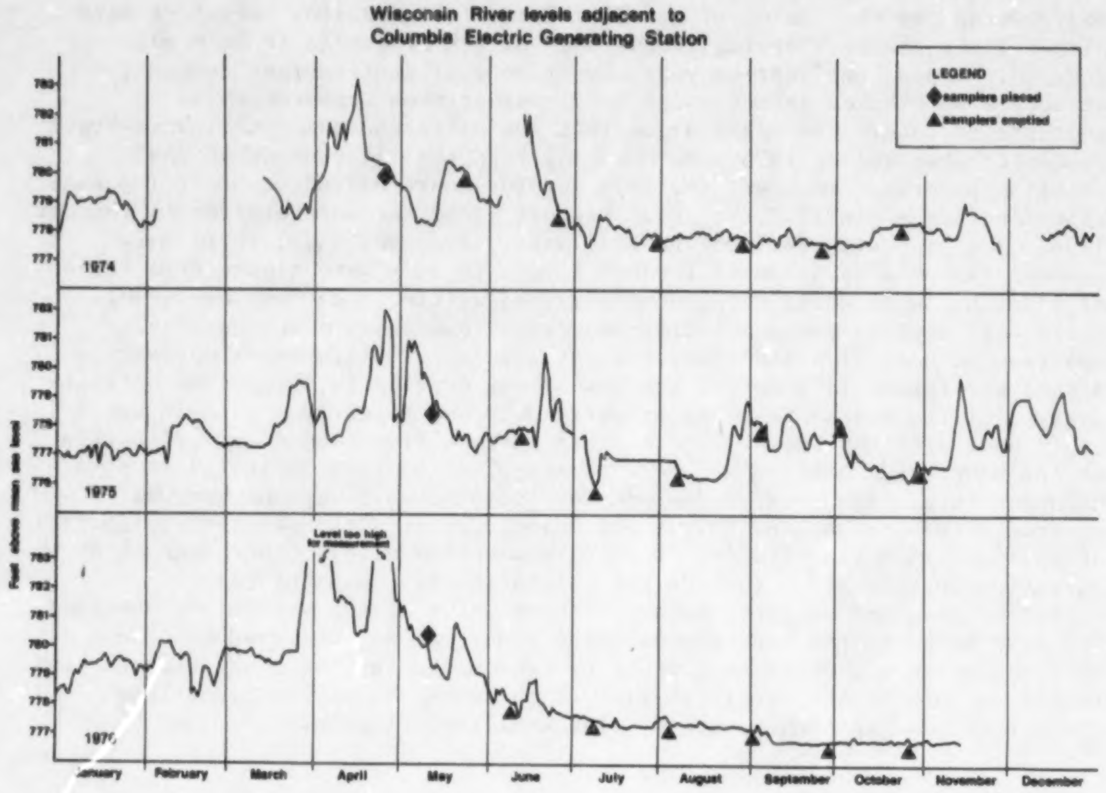

Figure 19. Water levels in the Wisconsin River at the Columbia Generating Station site during 1974-76 and times when basket-type artificial substrate samples were placed and emptied.

SECTION 4

EFFECTS ON INDIVIDUAL ORGANISMS

INTRODUCTION

This section documents studies initiated in 1976 and 1977 to examine the effects of the ash effluent on several invertebrate species. These studies include: 1) exposure of crayfish in cages upstream and downstream from the influence of the ash effluent; 2) follow-up measurements of metabolic rates and trace-element body burdens for the crayfish caged in the field; 3) a laboratory feeding study of the uptake and effects of chromium on crayfish; 4) laboratory exposures of amphipods and isopods to the ash effluent for data on survival, growth, and reproductive success; 5) an examination of spatial and temporal variability in life histories of mayfly nymphs collected in the study area. The mayfly study documented variability in seasonal cycles as part of the baseline data rather than as a study of specific effects of the ash effluent.

For the experiments on the effects of the ash effluent, three crustaceans that occur on the site and are easily handled in the laboratory were selected: an isopod, *Asellus racovitzai*; an amphipod, *Gammarus pseudolimnaeus;* a crayfish, *Orconectes propinquus*. *Asellus* is extremely abundant in the mint drain and Rocky Run Creek and is still present in low numbers in the ashpit drain. *Gammarus* is common to Rocky Run Creek, particularly at stations upstream of the old bridge abutment (R1, R2, R3, and R4) (Figure 1). Several species of *Orconectes* have been collected at the site. The crayfish were selected as a representative benthic detritivore large enough for trace-element analysis of individual tissues, for holding in field cages with large enough mesh to maintain current flow and food supply, and for convenient behavioral observations.

The Trace Elements and Aquatic Chemistry subprojects supplied data on heavy-metal and trace-element concentrations for various components and locations in the aquatic system (described in detail in Section I). These data established increased levels of several elements in aquatic invertebrates and in both soluble and particulate forms in the water column. Of particular interest were chromium and barium which were higher in the water column, suspended particulates, and organisms in post-operational years.

Considering the high concentrations of some elements in the particulate fraction of the affected waters (see Table 4), we have hypothesized that food might be an important source of trace-element exposure for benthic detritivores such as crayfish and isopods. At the same time, our knowledge of the forms and concentrations of all coal-combustion byproducts in the ash effluent has been incomplete. Thus it has been necessary to examine habitat modifications and organism responses caused by the ash effluent as a whole using conductivity as an estimate of the effluent concentration at any one time.

EXPOSURE OF CRAYFISH TO ASH EFFLUENT AND CHROMIUM-CONTAMINATED FOOD

Exposure of Crayfish to Ash Effluent

Introduction--

The ash basin effluent from the Columbia Generating Station contains elevated concentrations of metals in both soluble and particulate forms (Helmke et al. 1976a and 1976b, Andren et al. 1977). These increased metal levels might have adversly affected aquatic organisms inhabiting the drainage system. Organisms collected from the ashpit drain contained significantly higher concentrations of barium, chromium, selenium, and antimony than did organisms from unaffected sites (Schoenfield 1978). Although many trace metals are essential to organisms in small concentrations, exposure to high concentrations may interfere with important physiological processes. When organisms accumulate excess quantities, their ability to survive or maintain a population may be impaired.

The objectives of this study were: 1) to determine the effects on crayfish of exposure to a coal-ash effluent containing elevated metal concentrations and 2) to study the effects of the ingestion of chromium-contaminated food. Mortalities, metabolic rates, and tissue metal uptake were determined for crayfish exposed to ash effluent in the drainage system. Metabolic rate measurement may be a particularly valuable means of detecting sublethal effects since oxygen consumption can reflect many kinds of tissue damage or enzyme impairment. Ingestion may be an especially important mode of uptake for chromium and other metals because metals are in particulate form in the ashpit drain and are consequently available for consumption by detritivores such as crayfish. To assess the degree of metal uptake by detritus, and hence the quality of this food source for crayfish, leaf material was soaked at effluent-affected sites and analyzed for metal concentration.

Materials and Methods--

Crayfish collection and holding facilities--The crayfish, *Orconectes propinquus* (Girard), used in all experiments were collected from dense populations in Trout Lake, Vilas County, Wisconsin, with liver-baited minnow traps or by divers with hand nets. The crayfish inhabits streams affected by the generating station (Forbes, personal communication) but not in high enough numbers for intensive experiments. In the laboratory, crayfish were held in a flow-through system of three 190-liter glass aquaria and were fed trout pellets approximately twice weekly. Water temperature varied seasonally from 12 to 26°C; photoperiod was 16 h light:8 h darkness.

Water Chemistry Analysis--On-site measurements were made as follows: Dissolved oxygen was measured directly with a YSI Model 54A meter;

conductivity and temperature were measured with a YSI Model 33 meter; current speed was measured with a Midget C.M. Neyrpic current meter. Conductivities were corrected to 25°C. Water samples were collected with a Magnuson-Stuntz siphon (Magnuson and Stuntz 1970) and returned to the laboratory for determination of pH, hardness, alkalinity, and turbidity.

Laboratory analysis of field and laboratory samples was performed as follows: In the laboratory, pH was measured with a Fisher Accumet Model 150 meter; turbidity was measured with a Hach Model 2100A meter. Alkalinity was determined using the phenolphthalein and methyl orange indicator methods and hardness was measured with the EDTA titrimetric method (American Public Health Association 1976). Laboratory measurements of dissolved oxygen were from Broenkow and Cline's (1969) and Klinger's (1978) modifications to the Modified Winkler Method (American Public Health Association 1976).

Statistics--Symbols indicating levels of statistical significance are: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Unless otherwise indicated, means are reported with the standard deviation of the sample. Degrees of freedom are given with the symbol d.f. For analysis of variance, d.f. are given first for the numerator mean square, then for the denominator mean square.

Field Exposure--Six male and six female crayfish collected in June and August 1977 were caged at four sites in and near the ashpit drain (Figure 1) between 16 Sept. and 17 Nov. 1977. The two treatment sites were in the ashpit drain (A-4) about 3 km downstream from the ash basin and in Rocky Run Creek (R-4) immediately downstream of its confluence with the ashpit drain. The two control sites were in the mint farm drain (A-1) just before it merged with the ashpit drain and in Rocky Run Creek (R-2) just upstream from its confluence with the ashpit drain. Crayfish were caged in plastic minnow traps (Figure 20). One male and one female crayfish occupied each cage, one animal in each compartment. The cages, resting on the substrate in the middle of each stream, were anchored perpendicular to the flow with bricks. The soft sediment at each site partially filled the cages. Organic matter and organisms in the sediment flowed into the cages providing food for the crayfish. Cages were checked once or twice a week and animals found missing or dead before 22 Oct. were replaced.

Temperature and conductivity were measured at each site on every visit. Every other week current speed and dissolved oxygen were measured and water samples were collected for laboratory determination of pH, hardness, total and phenolphthalein alkalinity, and turbidity.

The generating station was shut down for maintenance from 3 to 19 Oct. 1977. During this time period, no ash effluent was pumped from the ashpit into the drainage system. Consequently, crayfish surviving the entire period were not as completely exposed to the effluent for 16 out of 62 days.

Respirometry--All crayfish were returned to the laboratory on 17 November and placed in closed system respirometers (Figure 21) randomly assigned to positions in a cold room. The crayfish acclimated for 24 h in continuously aerated water from their own caging sites. The water

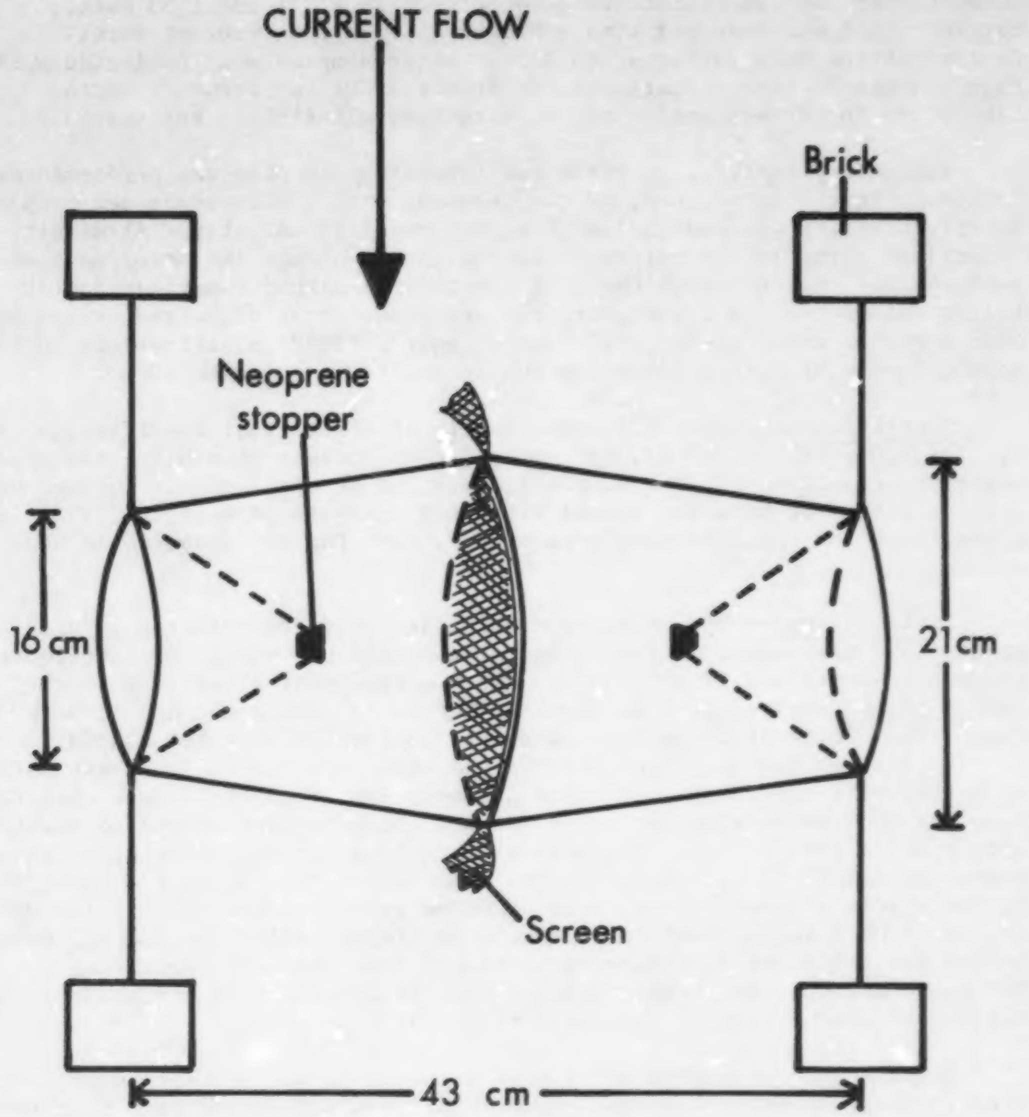
CURRENT FLOW
Brick
Neoprene stopper
16 cm
21cm
Screen
43 cm

Figure 20. Top view schematic drawing of the modified "Trophy" No. 20737 minnow trap used for caging crayfish at sites in the ash basin drainage system. Neoprene stoppers blocked each entrance funnel and a 30- × 30-cm piece of PVC-coated fiberglass screen divided each trap into two compartments. Trap mesh size ranged from 4 × 5 to 2 × 3 mm.

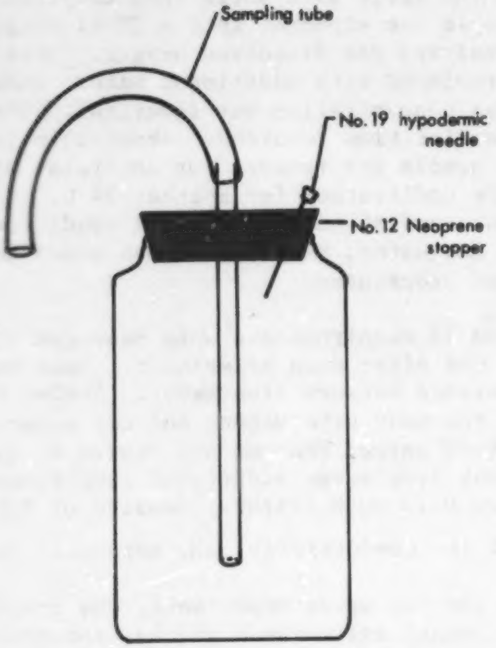
Sampling tube
No. 19 hypodermic needle
No. 12 Neoprene stopper

Figure 21. **Schematic drawing of the 465-ml glass jars used as respirometers (Klinger 1978). The sampling tube consisted of 15 cm of 5-mm I.D. glass tubing and 30 cm of 6.4-mm I.D. "Tygon" tubing. The stopper and sampling tube created an air-tight seal in the jar. The hypodermic needle inserted through the stopper allowed air to enter the top of the jar as water was siphoned from the bottom during sampling.**

temperature was 5°C and photoperiod was 10.5 h light: 13 h darkness, approximating November field conditions.

After crayfish were acclimated, airstones were removed and the water in each jar was replaced gently with aerated, filtered (0.45 Millipore filter) water from the field site where that crayfish originally had been caged. A water sample was siphoned into a 30-ml reagent bottle, stoppered immediately, and analyzed for dissolved oxygen. This water, taken from the respirometer, was replaced with additional water, under the assumption that the dissolved oxygen concentration was identical. The stopper was inserted securely and the initial time recorded. About 24 h later, time was recorded and a second water sample was removed for analysis. Airstones were provided and the animals were undisturbed for another 24 h. To determine metabolic differences between crayfish under identical conditions, all crayfish were placed in filtered tap water; respiration was measured immediately using the previously mentioned procedures.

Temperatures in 14 respirometers were measured with a mercury thermometer before and after each experiment. Mean temperature was 4.9 ± 0.5°C with no difference between treatments. Twelve respirometers with no crayfish contained the four site waters and tap water and served as controls to determine dissolved oxygen changes not caused by crayfish. Triplicate oxygen determinations from seven additional respirometers showed analytical error no larger than 0.13 mg O_2/liter. Samples of filtered site and tap water were analyzed for conductivity, pH, hardness, and alkalinity.

At the end of the tap water experiment, the crayfish were weighed, measured (carapace length and maximum width), and frozen in individual plastic bags. All animals were later dissected and analyzed for metal concentrations.

A stepwise multiple regression was performed separately on the oxygen consumption data (M = mg O_2 consumed/h) for experiments in site and tap water. Caging location and log wet weight were the only variables that related significantly to oxygen consumption. Sex and interactions between variables were not important. The regression coefficients, b, of the equations using site and log weight ($b = 0.751$ for site water; $b = 0.939$ for tap water) were used to find a weight-corrected metabolic rate for each crayfish in each experiment based on the assumption that $M = KW^b$ or $\log M = \log K + b \log W$ (where W = weight and K and b are constants) (Prosser 1973). The weight-independent metabolism, K (Prosser 1973), expresses the metabolic rate of a unit-sized organism (in this case 1 g). Since the relationship between weight and oxygen consumption is not linear, crayfish of different sizes will have different metabolic rates (V_{O_2} = mg O_2 consumed/h/g). Thus, metabolic rates should not be compared without first correcting for the effect of weight by determining weight-independent metabolism. This value can not be obtained by multiplying K by the weight. Instead, the equation $M = KW^b$ must be used. Log K was determined for each crayfish and the means for the groups were compared with analysis of variance and appropriate

a priori tests: [ashpit drain (A-4) against its control, mint drain (A-1); Rocky Run downstream (R-4) against Rocky Run upstream (R-2); pooled treatments (A-4 + R-4) against pooled controls (A-1 + R-2)].

Calculations were performed using crayfish wet weight. The relationship between wet weight and dry weight is shown in Appendix E. This relationship may be used to convert the individual metabolic rate data to a dry weight basis.

Soaking of Leaves in Ash Effluent--To assess the quality of effluent-exposed leaf material as a food source for crayfish, whole sugar maple (*Acer saccharum*) leaves were soaked at five sites in July 1977. Mint and ashpit drain sites were the same as those in the crayfish caging experiment (A-4 and R-4), but the Rocky Run Creek sites were 0.3 km below the confluence with the ashpit drain near the old bridge abutment and above the confluence at R-1 (Figure 1). An additional site, A-2, was also chosen immediately downstream from the confluence of the ashpit drain with the mint drain. Leaves in nylon mesh bags were anchored to the substrate with bricks and left in the water for 2 weeks, then returned to the laboratory for metal analysis. Samples were of two types: 1) a composite of leaves soaked at four separate time periods and dried; 2) a sample of leaves soaked during one time period and frozen. The leaf samples were analyzed by the University of Wisconsin nuclear reactor as described in the section on metal analysis.

Metal Analysis--Crayfish were dissected under a laminar flow hood using separate stainless steel tools for each tissue removed. Chrome-plated stainless steel has a high amount of chromium, so scissors and scalpels were used as little as possible to avoid metal-on-metal abrasion that might contaminate the samples. Instruments were carefully cleaned after each dissection by washing with "Micro" brand detergent and rinsing with a series of solutions: distilled water, distilled-deionized water, methanol, and distilled-deionized water. Exoskeleton, gill tissue, the hepatopancreas, and the abdominal muscle were removed and placed in separate preweighed 0.4-dram polyethylene vials, reweighed to obtain wet tissue weights, and oven dried at 40°C to obtain dry weights. Carcasses were placed in preweighed 30-ml jars and oven dried. Carcass dry weight and the four tissue dry weights were summed to obtain an approximate whole animal dry weight. Leaf samples were torn into small pieces with forceps, placed in 0.4-dram vials, and oven dried. Metal concentrations in exoskeleton and abdominal muscle were determined by the University of Wisconsin nuclear reactor using neutron activation. The neutron flux was 17×10^{12} neutrons/cm^{12}/sec. Gill samples were not analyzed.

Hepatopancreas samples were too small to be analyzed by the nuclear reactor. After drying, each hepatopancreas was transferred to preweighed high purity quartz tubing (1.5 mm inner diameter), reweighed, and heat-sealed. The following standards were prepared and placed in identical tubes: 1) synthetic liquid standard containing 40.0 µg/ml Cr, 60.0 µg/ml Ba, 30.0 µg/ml Sb, and 5.00 µ/ml Se in 0.4 *M* HNO_3; 2) National Bureau of Standards - Standard Reference Material #1571, orchard leaves; and 3) Canadian Certified Reference Materials Project, SO-4, Bottle 289 (described by Koons and Helmke 1978). These three standards were also included as samples with the tissues sent to the nuclear reactor to determine the accuracy of reactor analysis. This information is given in Appendix F. The hepatopancreas samples and standards were irradiated at the nuclear reactor with a neutron flux of 8 to 10 x 10^{12} neutrons/cm^2/sec and allowed to "cool" for approximately 2 weeks. The quartz tubes were then taped to the centers of posterboard cards to permit replication of sample geometry and each was radioassayed for approximately 6 h using two lithium-drifted geranium Li(Ge) detectors as described for the chromium ingestion experiment. A Tracor Northern Model TN-11 computer-based multichannel analyzer processed the signals (Koons and Helmke 1978).

Where metal concentrations were high enough, peak areas were calculated by computer, with adjustments for background and decay. In other cases, peak areas were calculated by the same method used for assays of the live crayfish in the chromium feeding experiment.

Although data on a large number of metals were obtained by both methods, only five metals (chromium, barium, zinc, selenium, and iron) were studied (Table 14). These were selected because of their elevated concentrations in the ash effluent or organisms (Helmke et al. 1976a, 1976b). When two energy peaks were obtained for the same metal, a mean concentration was calculated. Interference caused discrepancies in some samples and these data were discarded.

Results--

Effects of Ash Effluent on Water Quality--Ash effluent inputs to the mint drain and Rocky Run Creek resulted in consistent chemical and physical differences in water quality (Table 15). Conductivity was greatly increased in the ashpit drain (A-4) due to a high concentration of ions, principally sodium, and was also much higher in Rocky Run Creek downstream from the confluence with the ashpit drain (R-4). Alkalinity and hardness were much lower at the effluent-affected sites (A-4 and R-4) than at the control sites (A-1 and R-2). In the ashpit drain, pH was somewhat higher than in the mint drain and a very slight increase persisted at the downstream Rocky Run Creek site. The very high pH of the undiluted ashpit effluent is reduced before it reaches the mint drain by the addition of sulfuric acid. At other times of the year, pH values in the ashpit drain were consistently lower than in the mint drain.

Concentration of dissolved oxygen was higher in the ashpit drain than in the mint drain and was slightly lower at the downstream Rocky Run Creek site (R-4) than at the upstream site (R-2). Oxygen variation was probably due more to the effects of current speed than to the nature of the effluent itself. Current speed was much greater in the ashpit drain (A-4) than in the mint drain (A-1) because of its greater volume of water and was reduced in Rocky Run Creek downstream from the confluence with the ashpit drain (R-4). Groundwater inflow from the cooling lake slightly warmed the ashpit drain water (Stephenson and Andrews 1976). This temperature increase

TABLE 14. LIST OF METALS ANALYZED IN LEAF AND CRAYFISH TISSUE SAMPLES FOR THE CRAYFISH CAGING AND CHROMIUM INGESTION EXPERIMENTS[+]

Metal	Half life (days)	Gamma ray energy peak location(s) (KeV)
Soil Science Analyzers		
Chromium	27.8	320
Barium	12.0	496
Zinc	243	1,115
Selenium	120	265, 280
Iron	45.6	1,099, 1,292
U.W. Nuclear Reactor		
Chromium	27.8	320
Barium	12.0	216
Selenium	120	265
Iron	45.6	1,099
Zinc	243	1,115
Cadmium[‡]	2.2	528

[+] Hepatopancreas samples were analyzed using detectors at the University of Wisconsin Soil Science Department; all others were assayed at the University of Wisconsin nuclear reactor.

[‡] Cadmium was below the detection limit in almost all samples, except for some leaf material.

sometimes persisted in Rocky Run Creek. Turbidity was greatly increased in the ashpit drain, but was slightly higher at the downstream Rocky Run Creek site than at the upstream site.

The dilution of the ash effluent by Rocky Run Creek water w observed. The differences between control and downstream sites for all parameters measured were much smaller in Rocky Run Creek (R-2 and R-4) than in the ashpit drain system (A-1 and A-4). When the generating station was not operating, most parameters (conductivity, alkalinity, hardness, dissolved oxygen, current speed, and temperature) in the ashpit drain (A-4) and Rocky Run Creek downstream (R-4) returned to levels similar to those at their control sites (A-1 and R-2).

TABLE 15. CHEMICAL AND PHYSICAL PARAMETERS OF SITE WATER DURING CRAYFISH CAGING+

	Mint Drain	Ashpit drain (A-4)		Rocky Run upstream (R-2)	Rocky Run (R-4)	
		Pumping	No pumping		Pumping	No pumping
Temperature (°C)	11.2±4.04 3.8-16.2 13	12.80±4.50 4.8-17.5 10	11.60±4.07 9.2-16.3 3	10.99±3.91 3.6-14.8 12	11.60±4.18 3.8-16.2 10	9.33±1.88 8.2-11.5 3
Current speed (cm/sec)	5.9±3.6 3.5±11.1 4	25.5±12.8 13.4-38.9 3	7.4 --- 1	21.2±4.1 17.3-25.9 4	16.5±2.7 13.4-18.5 3	14.9 --- 1
Conductivity (μmhos/cm at 25°C)	457±12 438-476 13	1.844±439 1,209-2,443 10	553±121 482-693 3	527±62 479-636 12	900±210 672-1,290 10	529±87 473-630 3
Alkalinity, phenolphthalein (ppm)	0 --- 4	0 --- 4	0 --- 1	0 --- 4	0 --- 4	0 --- 1
Alkalinity, total (ppm)	232.52±18.37 211.07-251.00 5	100.20±12.12 90.60-114.50 4	227.81 --- 1	256.72±22.12 240.73-283.25 5	201.36±16.76 176.73-213.50 4	232.94 --- 1
Hardness (ppm)	253.18±1.56 251.60-255.37 5	167.05±19.39 148.19-190.41 4	244.00 --- 1	272.18±3.62 268.80-276.49 5	233.40±17.92 216.40-258.62 4	239.2 --- 1
pH	7.16±0.15 7.02-7.35 5	7.39±0.15 7.29-7.59 4	7.55 --- 1	7.65±0.09 7.52-7.75 5	7.73±0.09 7.60-7.79 4	7.75 --- 1
Turbidity (JTU)	6.2±2.6 4-13 4	18±5.6 13-24 3	17 --- 1	8.1±1.5 6-9.5 4	9.0±2.6 7-12 3	9.5 --- 1

+Separate analyses were performed for the ashpit drain (A-4) and downstream Rocky Run Creek (R-4) during the 16 days that no pumping occurred from the ashpit. Mean ± standard deviation, range, and sample size are given.

The filtered waters used in the respirometers were chemically similar to the unfiltered site waters (Table 16). The relative ordering of the sites was identical for conductivity, alkalinity, and hardness, and the differences for the pH measurements were minor. In the tap water, conductivity and alkalinity were similar to the control site waters and pH and hardness were higher than any of the site waters.

TABLE 16. CHEMICAL PARAMETERS OF WATER USED FOR MEASUREMENT OF METABOLIC RATES

	Site				
	Mint drain (A-1)	Ashpit drain (A-4)	Rocky Run upstream (R-2)	Rocky Run downstream (R-4)	Tap water
Conductivity (mhos/cm) at 25°C	446	1,519	484	734	521
pH	7.73	7.78	7.95	7.78	8.21
Phenolphthalein Alkalinity (ppm)	0	0	0	0	0
Total alkalinity (ppm)	217.28	93.70	248.51	176.73	217.47
Hardness (ppm)	252.13	149.41	273.64	212.74	304.09

Length of Exposure and Mortality--Three mortalities occurred during the caging experiment and eight individuals escaped from the cages (Table 17). Escaped and dead crayfish were replaced during the first 5 weeks. One control mortality took place in the first few days, while the two ashpit drain crayfish died during the last 2 weeks of exposure. Visual examination of the data indicated that the three ashpit drain crayfish with less than 42 days of effluent exposure were not consistently different from other ashpit drain crayfish in metabolic rate or metal concentration (Harrell 1978). Length of exposure was therefore not considered during further analysis.

Sublethal Effects--Metabolic Rate--For both experiments--site water and tap water--the order of the mean weight-independent metabolic rates from highest oxygen consumption to lowest was as follows: Rocky Run Creek upstream (R-2), mint drain (A-1), Rocky Run Creek downstream (R-4), and ashpit drain (A-4) (Table 18). Metabolic rates declined in all groups when they were transferred to tap water (Table 18). The groups had significantly different metabolic rates in tap water ($P = 0.036$) and approached significant difference in site water ($P = 0.060$) (Table 19, Row 1). In both

TABLE 17. SURVIVAL, LENGTH OF EXPOSURE TO ASH EFFLUENT, AND MORTALITIES AMONG CRAYFISH CAGED AT FIELD SITES+

	Survived until termination of experiment			Mortalities		
Site	No. of crayfish	No. of days caged on site	No. of days exposed to full ash effluent	No. of crayfish	No. of days survived	No. of days exposed to full ash effluent
A-1	5	62	0	1	4	0
	1	58	0			
	4	51	0			
	2	48	0			
A-4	7	62	46	1	55	39
	1	27	27	1	62	46
	2	48	32			
R-2	10	62	0	0		
	1	58	0			
R-4	12	62	46	0		

+Length of exposure is less than time caged due to plant shutdown for 16 days. Crayfish caged for less than 62 days (full length of experiment) were replacements for dead or escaped crayfish. All surviving crayfish were used for respirometry. Sites were at the mint drain (A-1), ashpit drain (A-4), Rocky Run Creek upstream (R-2), and Rocky Run Creek downstream (R-4).

experiments, the control crayfish (A-1 and R-2, pooled) had significantly higher metabolic rates than the effluent-exposed crayfish (A-4 and R-4, pooled) (Table 19, Row 2). Metabolic rates of mint drain (A-1) and ashpit drain crayfish (A-4) were significantly different in their site water, but not when they were transferred to tap water. There were no differences in metabolic rate between the two Rocky Run Creek sites (R-2 and R-4).

Some respirometers reached low levels of dissolved oxygen, leading to concern that stress caused differences in oxygen consumption (Larimer and Gold 1961, Wiens and Armitage 1961, McMahon et al. 1974). Final oxygen concentrations in the site water experiment ranged from 8.00 to 0.78 mg/liter, with five of the 44 respirometers less than 3.00 mg/liter. However, there was no difference in distribution of low oxygen respirometers between groups of crayfish (Analysis of variance, F = 1.089, n.s., d.f. = 3,40) and, therefore, relative differences in metabolic rate were probably not affected.

TABLE 18. WEIGHT-INDEPENDENT METABOLIC RATES (K = mg O_2 CONSUMED/H/1.0g SIZED CRAYFISH, WET WEIGHT) FOR CRAYFISH CAGED AT FOUR SITES[+]

Site	K (Mean)	Log K ± S.E.	Wet Weight (g)	No. of Samples
		Metabolism in site water		
R-2	0.03327	-1.478 ± 0.021	5.97 ± 1.24	11
A-1	0.03258	-1.487 ± 0.024	5.52 ± 2.05	12
R-4	0.02931	-1.533 ± 0.033	6.40 ± 2.30	
A-4	0.02483	-1.605 ± 0.063	5.92 ± 1.96	10
All crayfish			5.95 ± 1.89	44
		Metabolism in tap water		
R-2	0.02009	-1.697 ± 0.031	5.97 ± 1.24	11
A-1	0.01592	-1.798 ± 0.041	5.52 ± 2.05	12
R-4	0.01556	-1.808 ± 0.048	6.44 ± 2.20	12
A-4	0.01315	-1.881 ± 0.051	5.92 ± 1.96	10
All crayfish			5.96 ± 1.87	45

[+] The 1.0-g crayfish is a hypothetical unit-size crayfish. Mean wet weight, W ± S.D., and sample size are also given. Values of log K were used for statistical comparisons.

TABLE 19. DIFFERENCES IN METABOLIC RATES AMONG CRAYFISH CAGED AT TREATMENT AND CONTROL SITES. ANALYSIS OF VARIANCE AND *A PRIORI* TESTING WERE PERFORMED ON DATA COLLECTED IN WATER FROM THE CAGING SITES AND SUBSEQUENTLY IN TAP WATER[+]

Comparisons	F - ratio	
	Site water	Tap water
Analysis of variance	2.67 n.s. (d.f. = 3,40)	3.12* (d.f. = 3,41)
Treatments vs. controls (A-4 + R-4) vs. (A-1 + R-2)	5.937* (d.f. = 1,40)	4.871* (d.f. = 1,41)
A-4 vs. A-1	5.741* (d.f. = 1,40)	1.945 n.s. (d.f. = 1,41)
R-4 vs. R-2	1.258 n.s. (d.f. = 1,40)	3.660 n.s. (d.f. = 1,41)

[+] Degrees of freedom are given for the numerator mean square and the denominator mean square.

Nine of the 12 empty respirometers gained oxygen, resulting in a mean gain of 0.15 ± 0.37 mg O_2/respirometer for the site water experiment. This gain was randomly distributed among types of water (Kruskal-Wallis Test, H = 4.79, n.s. d.f. = 3); therefore, it is unlikely to have biased the results.

Metal Uptake--Crayfish exposed to ash effluent accumulated all metals studied, but tissues differed in the degree to which they acquired the metals (Figure 22). Chromium was located primarily in the hepatopancreas, but there were smaller amounts in muscle and exoskeleton samples. Barium appeared in the hepatopancreas and exoskeleton, selenium in the hepatopancreas and muscle, and iron in the hepatopancreas. Iron in the exoskeleton was the only element significantly lower in the ashpit drain as compared to the mint drain. Zinc occurred in all three tissues analyzed. The analytical method did not distinguish between metals adsorbed onto the exoskeleton surface and those actually assimilated into the tissue. Taking this into consideration, the hepatopancreas incorporated the greatest concentrations of most metals, with the exception of zinc, which was highest in muscle. These results were quite similar to those obtained from tissue analysis of the chromium-fed crayfish (see Table 24).

Differences in tissue metal concentrations among the four crayfish groups were usually significant (Table 20, Col. 1). Only chromium in the muscle and barium in the hepatopancreas did not show differences. Results of the *a priori* tests (Table 20, Cols. 2 through 4) were more variable. The only difference between the two Rocky Run Creek crayfish groups was the higher chromium in the hepatopancreas of the downstream group (Table 20, Col. 4). Effluent-exposed crayfish (A-4 and R-4, pooled) had higher metal concentrations in most cases than did the controls (A-1 and R-2, pooled) (Table 20, Col. 2). This difference between controls and treatments appeared to be caused by the elevated metal levels in ashpit drain crayfish as compared to their controls in every case except for chromium in the hepatopancreas (Col. 3). Even in those tissue-metal combinations where most values were below the detection limit, any values above the limit were usually ashpit drain samples (Table 20). This may indicate that with low enough detection limits, a significant difference between crayfish groups might have been found.

Leaves soaked at effluent-exposed sites generally had higher concentrations of chromium, barium, and selenium than the control site leaves (Table 21), with the downstream Rocky Run Creek (R-4) leaves higher than those from the ashpit drain (A-4). Values for iron were similar at the control (A-1) and Rocky Run Creek (downstream of A-4) sites but were lower in the ashpit drain (A-4). There was no obvious pattern for zinc. Differences between single and composite samples could be due to seasonal variations or to the fact that single samples were frozen before they were dried.

Discussion--

Mortality--Exposure to the effluent had no significant lethal effects. Total mortality during the exposure of the crayfish to ash

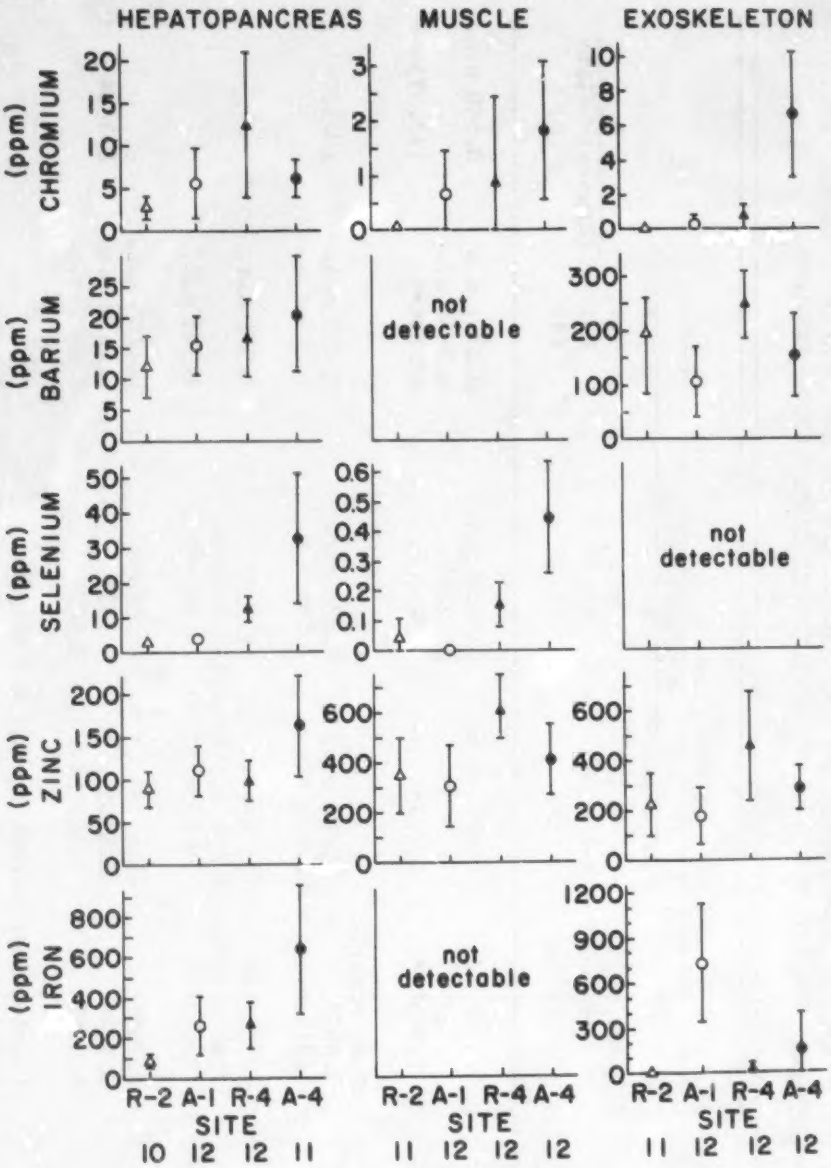

Figure 22. Concentrations of five metals in the tissues of crayfish caged at four sites. Means are given in ppm (dry weight) with 95% confidence intervals. Where individual samples were below the detection limit, they were assigned a concentration of 0 ppm and included in the calculation of the mean. Sites are shown in order of increasing effluent concentration, with R-2 and A-1 as control sites with no effluent. Sample sizes are listed below the site names.

○ = A-1 (mint drain) △ = R-2 Rocky Run Creek Upstream

● = A-4 (ash pit drain) ▲ = R-4 (Rocky Run Creek Downstream

TABLE 20. SIGNIFICANCE TESTS FOR DIFFERENCES IN TISSUE METAL CONCENTRATIONS IN CRAYFISH CAGED AT FOUR SITES. WHERE THE DATA APPEARED NOT TO BE NORMALLY DISTRIBUTED (CHROMIUM IN MUSCLE AND HEPATOPANCREAS, BARIUM AND ZINC IN EXOSKELETON), A NON-PARAMETRIC ANALYSIS OF VARIANCE (KRUSKAL-WALLIS TEST) GAVE THE SAME DEGREE OF SIGNIFICANCE AS DID ANALYSIS OF VARIANCE, AN INDICATION THAT THE ANALYSIS WAS NOT AFFECTED BY DISTRIBUTION PROBLEMS

	F - ratios				
		A priori Tests			
Metal and tissue	Analysis Variance (1)	A-4 + R-4 vs. A-1 + R-2 (2)	A-4 vs. A-1 (3)	R-4 vs. R-2 (4)	H (Kruskal-Wallis (5)
Cr in muscle	0.4557 n.s.	0.8106 n.s.	0.5958 n.s.	0.2711 n.s	0.550 n.s.
Cr in exoskeleton	13.265***	16.53***	27.22***	0.3488 n.s.	
Cr in hepatopancreas	12.986***	4.777*	0.0290 n.s.	22.89***	147.075***
Ba in muscle	Undetectable				
Ba in exoskeleton	3.897*	3.008 n.s.	1.336 n.s.	1.455 n.s.	10.523*
Ba in hepatopancreas	1.317 n.s.	2.474 n.s.	1.536 n.s.	1.313 n.s.	
Se in muscle	17.042***	32.69***	42.33**	2.574 n.s.	
Se in exoskeleton	Undetectable except in three A-4 samples				
Se in hepatopancreas	10.672***	19.759***	24.450***	2.343 n.s.	
Zn in muscle	4.708**	8.612**	12.085**	0.4715 n.s.	
Zn in exoskeleton	3.474*	6.910*	9.131*	0.4870 n.s.	8.259*
An in hepatopancreas	4.039*	3.108 n.s.	5.447*	0.1472 n.s.	
Fe in muscle	Undetectable except in one A-4 sample				
Fe in exoskeleton	10.097***	7.306*	14.454***	0.206 n.s.	
Fe in hepatopancreas	7.367***	9.824**	10.386**	2.225 n.s.	

TABLE 21. CONCENTRATIONS OF METALS IN SUGAR MAPLE LEAVES SOAKED AT FIVE SITES IN THE ASH BASIN DRAINAGE SYSTEMS[+]

Site	Kind of sample	Metal concentration (ppm dry weight)				
		Ba	Cr	Fe	Se	Zn
A-1	Single[†]	175.1	10.78	14,080	b.d.	417.3
	Composite	122.0	7.49	5,981	0.1482	217.4
A-4	Single[†]	355.6	39.73	1,795	1.1060	245.2
	Composite	176.8	14.35	3,938	0.4981	238.8
0.3 km downstream from R-4	Single[†]	371.6	51.93	12,720	0.9121	244.5
	Composite	266.6	28.49	6,524	0.9658	193.7
R-1	Single[‡]	121.4	14.73	5,933	b.d.	336.0
	Composite	197.6	23.84	1,690	0.8892	237.6

[+] Single samples consisted of leaves frozen after one 2-week soaking period (Removed 20 July 1977). Composite samples consisted of leaves from several 2-week soaking periods (15 and 23 June, 1 and 7 July 1977).
[†] These were dried and combined for analysis. b.d. = below detection limit.
[‡] Single sample treated as above for 20 July. Composite sample from 1 and 7 July only.

effluent was low. The only non-ashpit drain death was in the mint drain and was possibly due to poor initial health of that particular crayfish. Both additional deaths occurred in the ashpit drain near the end of the experiment and may indicate the beginning of a trend, but sublethal effects most likely will limit the crayfish population in the ashpit effluent.

Metal Uptake--Crayfish living in the ashpit drain accumulated all of the metals studied (chromium, barium, zinc, selenium, and iron). By the time the effluent is diluted by Rocky Run Creek, environmental metal concentrations are reduced, and with the exception of chromium, the elements do not appear in the crayfish tissues in statistically significant amounts. It appears anomalous that chromium concentrations in the hepatopancreas were elevated over control levels in downstream Rocky Run Creek crayfish (R-4), but not in ashpit drain crayfish (A-4). Helmke et al. (1976a) reported that chromium concentrations in suspended particulates in the ashpit drain increase with distance from the ash basin. If this tendency toward increased chromium precipitation continues in Rocky Run Creek, there may be more chromium available to crayfish in the Rocky Run Creek sediments than in the ashpit drain. The higher chromium concentrations in effluent-exposed leaves from Rocky Run Creek than in those from the ashpit drain further support this hypothesis.

The five metals accumulated in different body tissues. All except barium were found in the hepatopancreas and all but selenium were found in the exoskeleton; selenium and zinc were also found in the abdominal muscle. These data do not indicate whether metals observed in exoskeleton samples are due to surface adsorption or to actual tissue incorporation. Schoenfield (1978) discussed this in detail and suggested using metal:scandium ratios to answer this question. He found that whole *Asellus racovitzai*, a benthic detritivore from the ashpit drain, had high levels of scandium indicating inorganic contamination. Ingestion of sediment hydrous iron oxides precipitated on the body surface could cause this contamination. Thus, the high concentrations of many metals in crayfish exoskeletons may be largely due to surface adsorption rather than tissue assimilation.

The importance of this surface contamination as a route for metal incorporation in other tissues is unclear. Exoskeleton permeability is a significant factor in metal assimilation by crustaceans and polychaetes (Bryan and Hummerstone 1973), but the crayfish exoskeleton may be relatively impermeable to some metals (Wiser and Nelson 1964). In either case, a tendency for metal adsorption to the surfaces of organisms indicates a potential source of metal contamination for crayfish in the ash effluent drainage system, whether from direct transport through the integument or from ingestion of surface deposits on detritus and prey organisms.

The high concentration of chromium in the hepatopancreas agreed with most findings reported in the literature (discussed in the section on chromium concentrations in laboratory-exposed crayfish). The hepatopancreas is important in dynamics and storage of many other metals as well, such as lead and copper in *Asellus* (Brown 1977), copper and zinc in the shrimp, *Crangon* (Bryan 1971), zinc in crabs, lobsters, and freshwater crayfish (Bryan 1966, 1967), and cobalt in crayfish (Wiser and Nelson 1964). Luoma (1976) found that mercury concentrations in the crab, *Thalamita crenata*, were higher in the viscera than in the body muscle. Thus, the hepatopancreas has a most important function in storing excess amounts of metals taken into the body and releasing them to other tissues or excreting them.

It is important to compare the data with Schoenfield's (1978) data on metal concentrations in organisms collected in the Columbia ash effluent system. Tissues of similar physiological function in frogs, *Rana pipiens*, and crayfish were similar in metal concentrations (Table 22). The only order-of-magnitude discrepancies were for selenium in the liver and hepatopancreas, and for zinc in the muscle. This may be due to different physiological mechanisms for dealing with metals in an amphibian vs. a crustacean or to differences in the amount of time animals were exposed to the ash effluent. Crayfish fed chromium in the laboratory also contained similar tissue chromium concentrations. With the exception of high zinc in the crayfish muscle, both studies implicate the hepatopancreas and liver as tissues that concentrate metals. This is expected when the function of these organs in metal detoxification, storage, and elimination is considered.

TABLE 22. MEAN METAL CONCENTRATIONS IN TISSUES OF FROGS, CAGED CRAYFISH, AND LABORATORY CRAYFISH[+]

	Liver (frogs) or Hepatopancreas (crayfish)	Muscle
Chromium (Cr)		
Frogs	4.9	1.9
Caged crayfish	6.2	1.8
Laboratory crayfish		
Cr[§]	2.3	‡
Cr[¶]	5.9	1.9
Barium (Ba)		
	15	‡
Caged crayfish	20	‡
Iron (Fe)		
Frogs	770	30
Caged crayfish	640	
Selenium (Se)		
Frogs	2.3	0.9
Caged crayfish	33	0.4
Zinc (Zn)		
Frogs	106	21.4
Caged crayfish	163	625

[+] Frogs were collected from site A-5 in Figure 1 (Schoenfield 1978). Caged crayfish were collected from site A-4 in Figure 1.

[‡] Indicates metal concentration below the detection limit.

[§] Crayfish in the laboratory exposed to Cr.

[¶] Crayfish in the laboratory exposed to ^{51}Cr-labeled Cr.

Ash Effluent Characteristics Affecting Crayfish Metabolism--Long-term exposure to effluent in the ashpit drain reduces the metabolic rate of crayfish and this reduction is more pronounced before further dilution occurs in Rocky Run Creek. There are three ash effluent characteristics that might play some role in the decreased metabolic rate of crayfish held in the ashpit drain and Rocky Run Creek below the confluence: Increased ionic concentration, reduced food supply, and increased heavy metal concentration. These parameters are among those frequently found to alter metabolic rates (Wiens and Armitage 1961, Vernberg et al. 1973, Rice and Armitage 1974, Frier et al. 1976, Nelson et al. 1977). Other important factors such as time of day, season, and activity were constant among treatments. Differences in weight were corrected for and differences in sex did not affect metabolic rate.

The increase in conductivity at sites receiving ash effluent may appear to explain the variations in metabolic rates of the crayfish based on both the substantial conductivity increase in the ashpit drain and on the widely reported effects of salinity changes on metabolism. However, a comprehensive study of the experimental results and the literature suggests that increased metal levels and decreased food supply are more valid explanations. Conductivity is important primarily as an indicator of the concentration of an ash effluent containing coal combustion byproducts, including trace elements, organic contaminants, fly-ash particles, and salts.

Recent work on the effects of environmental variables on invertebrate metabolic rates has involved marine or brackish water species. Although ionic composition may differ, salinity and conductivity are both expressions of ion concentrations. Metabolic rate changes in the ash effluent might be compared with results obtained in sea water, particularly since the high conductivity of the effluent is due primarily to sodium ions, a major ionic component of sea water. There is disagreement over the effects of salinity on metabolic rate (Nelson et al. 1977). Some authors suggest that increased metabolic rates at salinities differing from the organism's isosmotic point (point of equal osmotic pressure) indicate an increased energy cost due to osmoregulation (regulation of osmotic pressure in the body of an organism). Others report either a decrease in metabolic rate in non-optimal salinities, or else no correlation between them. The work of Nelson et al. (1977) with the prawn, *Macrobrachium rosenbergii* indicates a reduced metabolic rate when certain salinity levels are exceeded at various temperaures. Frier (1976) notes a marked increase in isopod oxygen consumption in low salinity water and Taylor et al. (1977) report the same increase for marine crabs, *Carcinus maenas*, exposed to 50% seawater at 10°C. Taylor et al. (1977) also found that this increase did not occur at 18°C and attributes this to quiescence and failure to osmoregulate in warmer waters. In the cooler water, however, the crabs used oxygen through osmoregulation and hyperactivity--possibly an avoidance mechanism. Vernberg et al. (1973) also report a decline in metabolic rate in less optimal temperature and salinity conditions for larval crabs, *Uca pugilator*.

Thus, in many marine species, oxygen consumption is lowest in solutions isosmotic with the blood. This can perhaps be extended to freshwater organisms that must continually use oxygen to osmoregulate. In an environment with higher conductivity, the water may be closer to the osmotic level of the blood and, consequently, less energy would be needed for osmoregulation. If this is true, the ashpit drain may be a beneficial environment rather than a hazard; however, there are reasons to doubt this. Most freshwater animals can not tolerate high salt concentrations, especially when only one salt is present (Hynes 1960), as is the case for the sodium in the ash effluent. The increased ionic content could adversely affect the organism's osmotic balance. If this reduced enzyme activity or impaired ability to obtain and transport oxygen, a lower rate of oxygen consumption would result. Prolonged exposure could cause cellular starvation or hypoxia and death could occur. Some of the data indicate that the mechanism of increased conductivity resulting in decreased metabolic rate is not entirely applicable. In the mint drain where the lowest conductivity is found, crayfish do not have the highest metabolic rates; crayfish from the Rocky Run Creek control site do. In addition, the ashpit drain and downstream Rocky Run Creek crayfish respond to tap water of lower conductivity with lower oxygen consumption rather than the hypothesized increase. The reduction in metabolic rate in tap water instead may be a response to the stress of acclimating to a new chemical environment. Further evidence of stress lies in the change in the regression coefficient for the relationship between log weight and log O_2 consumed/h. The value of b in site water (crayfish of all treatments pooled), 0.751, agrees well with the reported values near 0.74 (Prosser 1973). When exposed to tap water, b is considerably higher--0.939. Vernberg and Vernberg (1969) report that the value of b may change with temperature, salinity, environmental history, or geographic population. If the change from site to tap water is indeed a stress to the animal, the value of b as well as the metabolic rate could be considerably altered while the organism acclimates to its new environment.

In summary it appears that conductivity does not control metabolic rate, but indicates changes in the concentration of the ashpit effluent as it progresses downstream, an effluent that contains some other factor(s) causing reduced metabolism in crayfish. Decreased food supply and increased metal concentrations are potential causes of the changes in oxygen consumption.

No attempts were made to assess quantity and quality of food available for crayfish at the various sites. However, from visual observations, it appeared that quantity and perhaps quality are much lower in the ashpit drain than at the other sites. The most food is available at the upstream Rocky Run site followed by the mint drain. The mint drain is a narrow drainage ditch with much overhanging vegetation, primarily sedge grasses, and considerable duckweed, *Lemna*, on the surface. Rocky Run Creek drains a marsh system but it also receives detritus from macrophyte beds and floodplain forest. Both the mint drain and Rocky Run Creek have dark brown sediments with high organic content. The ashpit drain, in contrast, is diked for all of its length upstream from the caging site. There is little overhanging bank vegetation, and even less aquatic vegetation. The current is rapid, possibly removing detritus from the area. The sediment is lighter brown with more sand and contains much less organic matter. Higher turbidity may reduce photosynthetic activity. This reduces habitat diversity for aquatic vegetation and for animals that crayfish eat. High metal concentrations may reduce photosynthetic activity in plants, further reducing the food supply in the ashpit drain and downstream Rocky Run Creek. Clendenning and North (1960) found a 50% reduction in kelp, *Macrocystis pyrifera*, photosynthesis when 5 ppm of hexavalent chromium was added to the water.

Insufficient food may explain the deaths of two ashpit drain crayfish after 2 months at the site. Reduced food supply may also result in lower oxygen consumption. In a review of the literature, Newell (1973) reports that starvation is associated with reduced metabolic rate in many intertidal invertebrates. Lower feeding rates may reduce total activity levels (hence, lower metabolic rates). This may be an adaptation to use less energy when less food is available. The highest concentrations of chromium, barium, and selenium occur in leaves soaked at the two effluent-affected sites (A-4 and R-4); therefore, the quality of the food may be lower at these sites as well.

The third explanation suggested for the reduced metabolic rates at contaminated sites is the exposure of the crayfish to heavy metals. Although many trace metals are essential in small concentrations, such as in enzyme complexes and respiratory pigments, exposure to high concentrations of these metals may interfere with a wide variety of physiological processes. These effects have been extensively documented in the literature (Becker and Thatcher 1973, Eisler 1973, Eisler and Wapner 1975). Metals may decrease oxygen consumption by interfering with enzymes and oxygen transport molecules, resulting in a reduced ability to utilize oxygen. Cellular metabolism may become less efficient. Effects of metals on the gills may lead to reduced oxygen exchange capabilities. De Coursey and Vernberg (1972) found a reduced metabolic rate in *Uca pugilator* larvae upon exposure to 0.18 ppm of mercury. Vernberg et al. (1973) determined that 1.8 ppm of mercury reduced metabolism at 25° and 30°C and increased it at 20°C. They concluded that suboptimal conditions of temperature and salinity reduced metabolic rate and that the direction of the added stress from the mercury was temperature dependent. Fromm and Schiffman (1958) exposed largemouth bass to hexavalent chromium and observed reduced oxygen consumption after a brief initial increase. They attribute this to a gradual decrease in cellular metabolism caused by chromium accumulation in various tissues, rather than to direct impairment of respiration. They found no significant change in the respiratory epithelium or in opercular movements. Two species of crabs, *Macropoda rostrata* and *Pachygrapsus marmoratus*, consumed less oxygen when exposed to chromium (Chaisemartin and Chaisemartin 1976). Therefore, it appears that high metal levels in the ash effluent reduced crayfish metabolic rates by becoming incorporated into tissues and interfering with cellular metabolism.

Visual food supply assessment and concentrations of three metals in the hepatopancreas (chromium, selenium, and iron) follow the same ranking as does metabolic rate of crayfish exposed to those factors in ash effluent. Metabolic rate decreased from sites R-2 to A-1 to R-4 to A-4, as did food supply. Hepatopancreas metal levels increase in this same order. Thus, both factors probably interact to reduce the desirability of the effluent-affected habitats for crayfish. The metals in the effluent may be the ultimate cause of the observed sublethal effects, responsible not only for direct effects on crayfish metabolism, but also for the reduced food supply available to the crayfish. Severely reduced animal populations in the ashpit drain were observed after the power plant began operating (See Section 3). Animal material that may be a significant portion of the crayfish food supply in the non-contaminated environments would now be limited in quantity in the ashpit drain.

The modification of the mint drain by the addition of ash effluent has reduced its value as a habitat for crayfish, as well as for many other species. Heavy metal inputs, reduced food supply, altered ionic composition, turbidity, and the precipitation of barium and aluminum flocs all play some role in the impairment of a sizeable portion of the sedge meadow drainage system. This area is of particular importance to man as a spawning area for game fish (Priegel and Krohn 1975, Magnuson et al. 1980).

Although dilution of the effluent by ground and surface water substantially reduces the amount of contamination in Rocky Run Creek, the sublethal effects observed in the ashpit drain persist there. Metabolic rate was lower and metal concentrations were higher in crayfish from downstream Rocky Run Creek than in crayfish caged at the upstream control site. Even though these differences were not statistically significant (except for chromium in the hepatopancreas), long-term sublethal effects in Rocky Run Creek may gradually become apparent.

Other organisms and life cycle stages may be much less tolerant of environmental impairment than are mature crayfish. Juvenile crayfish are more susceptible to metal contamination than the adults tested in this experiment (Doyle et al. 1976, Hubschman 1967, Van Olst et al. 1976). Young-of-the-year *Gammarus* were more susceptible to ashpit drain water than were adults (See Experiment II, page 92). Eggs, juveniles, and recently molted individuals may have more permeable surfaces or less efficient metal storage and elimination systems. The crayfish, *Orconectes propinquus*, which is resistant to some metals--i.e., cadmium (Gillespie et al. 1977)--is important in food chains (Neill 1951) and may contribute significant amounts of metal to less tolerant organisms at higher trophic levels. Thus, the effects of metals on other organisms inhabiting the sedge meadow drainage system may be much greater than the effects observed in the adult crayfish caged in the effluent.

Exposure of Crayfish to Chromium-Contaminated Food

Introduction--

Waters receiving ash basin effluent have elevated concentrations of barium and chromium in the suspended particulate fractions (Helmke et al. 1976a). In addition, concentrations of barium, chromium, selenium, and antimony in organisms collected from the ashpit drain are much higher than in those from unaffected sites (Schoenfield 1978). Crayfish caged at effluent-exposed sites accumulate significant amounts of chromium, barium, selenium, iron, and zinc, and it is suspected that ingestion of high concentrations in particulate forms contributes substantially to the organisms' body burdens of the metals. Chromium was fed to crayfish in the laboratory to determine metal uptake and tissue accumulation, as well as mortality and sublethal behavioral effects.

Materials and Methods

Crayfish collection in September 1976 followed the procedures of the crayfish caging experiment and the animals were held in the laboratory under the same conditions until the experiment began. Crayfish were divided into three experimental groups. The first group (designated Cr*) was fed leaf discs soaked in a 1.0-ppm solution of chromium in distilled water that included a tracer amount of chromium-51. The second group (designated Cr) was fed leaf discs soaked in a 1.0-ppm chromium solution with no radioactive tracer. This group served as a control to detect any effects due solely to radiation and not to the chromium. The third group of crayfish served as controls (designated C) and were fed leaf discs soaked only in distilled water.

Food preparation--In October 1976, yellow leaves were removed from a single sugar maple tree, *Acer saccharum*, growing along the shore of Lake Mendota, Dane County, Wisconsin. They were oven dried for 3 days at 40°C and stored at room temperature in large polyethylene bags. After soaking leaves in water for 10 min to soften, a cork borer was used to cut discs 1 cm in diameter. Only entire discs without parts of the three primary veins were used. Discs were dried at 40°C and stored in covered glass jars.

A 1.0-ppm chromium solution was selected for leaf soaking because accumulation of chromium occurred and the concentration leveled off within 2 weeks (Figure 23). Discs soaked in 0.1 ppm chromium accumulated very little chromium, and those soaked at 50 ppm chromium had not reached a stable concentration after 3 weeks of soaking. It was suspected that allowing the concentration to stabilize would reduce the variation between individual discs. Two weeks appeared to be the optimal duration, since leaf matter may reach its maximum nutritive value for the detritivore, *Tipula*, after 2 weeks of stream conditioning (Cummins 1974). Leaf discs were pre-leached in Lake Mendota water because this procedure nearly doubled chromium uptake (Figure 23).

The food supply for each week was prepared by soaking 300 discs for each crayfish group in 500 ml of Lake Mendota water for 1 week, with water changes after 2 and 4 days. Discs were transferred to the appropriate soaking solutions: 500 ml of distilled water, 500 ml of distilled water containing 1 ppm chromium (as potassium chromate, $K_2 Cr O_4$), and 500 ml of 1 ppm chromium to which 80μCi of chromium-51 was added (obtained from the University of Wisconsin Radiopharmacy as 2 μCi ^{51}Cr in 1 ml of saline solution). The chromium solutions contained sufficient chromium atoms to allow each disc to attain maximum concentration. After 2 weeks in the treatment solution, each set of 300 discs was dried at 40°C for 2 days and stored in a separate glass jar. Before feeding to the crayfish, an appropriate number of discs was placed in 200 ml of distilled water on a magnetic stirrer for 1 h so that they would sink and be within reach of the animals.

Holding and feeding procedures--In February 1977, 1 month before beginning the experiment, six male and six female crayfish for each treatment began acclimating to the experimental aquaria and feeding procedures. Three males and three females were placed in each of six 38-liter glass aquaria. A Nytex screen divided each aquaria in half. An airstone was placed in each half in a perforated PVC plastic tube with a cotton plug at the top to prevent breaking air bubbles from spraying radioactive chromium into the air. Three short lengths of opaque PVC pipe

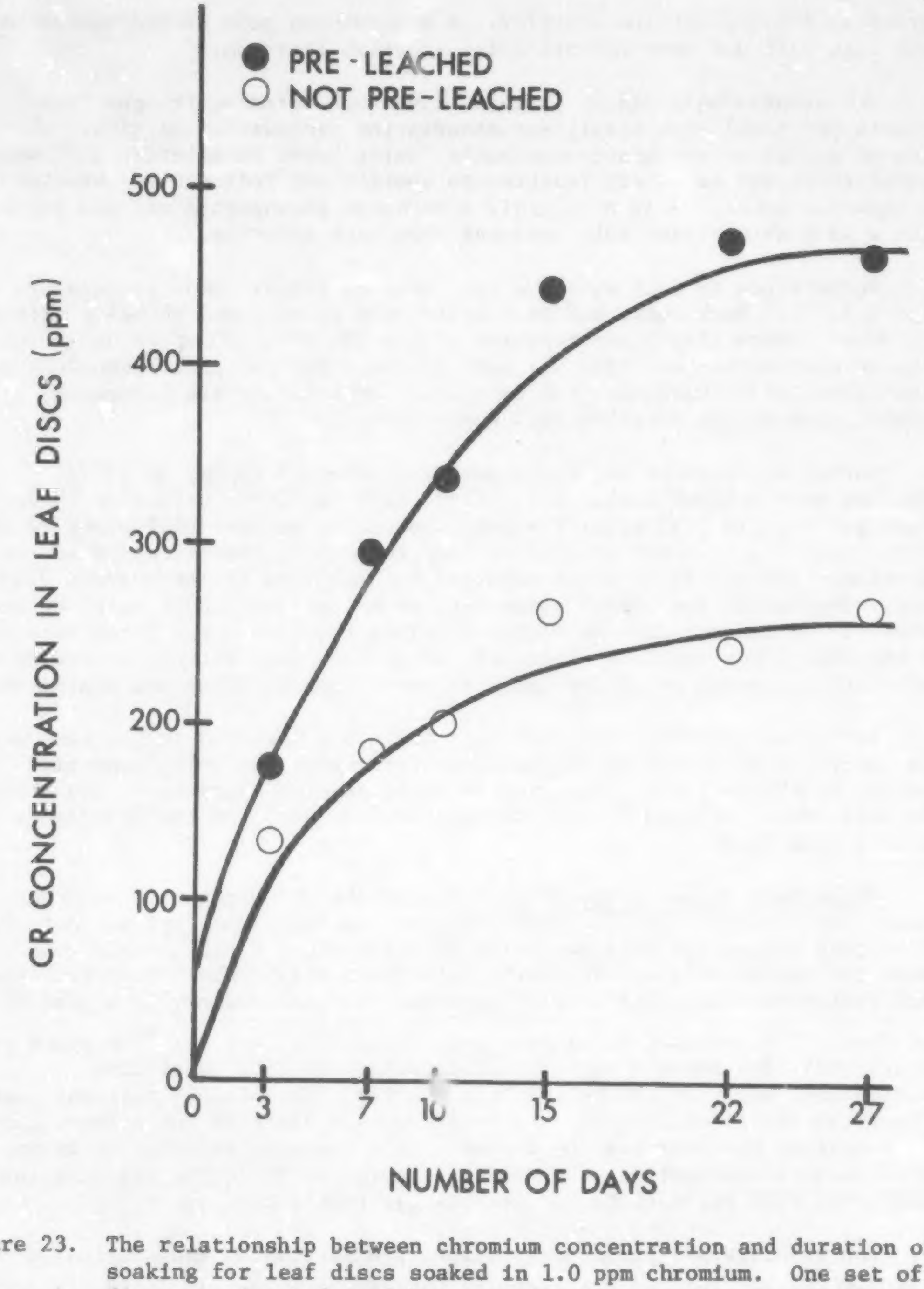

Figure 23. The relationship between chromium concentration and duration of soaking for leaf discs soaked in 1.0 ppm chromium. One set of discs was leached in lake water for 1 week prior to treatment. Discs used in the crayfish feeding experiment contained 263 ppm Cr (95% confidence limits: 172 to 354 ppm).

served as shelter for the crayfish. A nine-square grid on the bottom of each tank half was used for recording crayfish location.

The aquaria were placed in three livestock watering troughs (two aquaria per tank) with plexiglass observation windows on one side. The troughs served as thermistor-controlled water baths to maintain 20°C water temperatures and as safety features to contain any radioactive chromium if an aquarium broke. A 12 h light:12 h darkness photoperiod was maintained with a 15-W white light bulb centered over each aquarium.

Temperature in each aquarium was recorded daily. Mean temperature was 21.6 ± 1.3°C. Each week, before cleaning the aquaria and changing half of the water, conductivity was measured with a YSI model 33 meter and water samples were collected. Samples were analyzed for pH, phenolphthalein and total alkalinity, hardness, and dissolved oxygen using the laboratory methods used in the crayfish caging experiment.

During acclimation and the experiment (March 7 to May 12 1977), crayfish were weighed weekly and individually fed five leaf discs three times per week in 0.47-liter (1-pint) freezer containers with water at about 20°C. Each crayfish was allowed to feed for 1.5 h, then returned to its aquarium. The number of discs consumed was recorded to the nearest 0.25 disc. Feeding in individual containers permitted the amount eaten by each animal to be determined. In addition, since chromium could leach back out of the leaf discs into the water, the discs were kept out of the aquarium water and the exposure of the crayfish to soluble chromium was minimized.

During acclimation, crayfish were fed discs prepared in the same way as the control diet. Most of the male crayfish never fed well, possibly because they were too confined in the small freezer containers. Thus, all the males were replaced by randomly selected females and the experiment began 1 week later.

Whole-body chromium assay--Crayfish in the Cr* group were analyzed weekly for chromium uptake. Total chromium concentration was not determined since this method can only determine chromium added to the sample over and above the amount present before the experiment began. Whole-body radioassays were performed using high resolution gamma ray spectroscopy on a (Ge(Li) detector. The detector had a resolution of 2.0 KeV for the ^{60}Co gamma ray at 1.33 KeV. The signals were routed to a Nuclear Data Model 2200 multichannel analyzer (Koons and Helmke 1978). Chromium-51 releases gamma rays at an energy of 320 KeV. By assaying each crayfish for a known time and comparing the peak size to the peak of a standard solution of known chromium-51 concentration, the amount of chromium-51 in the crayfish was determined with appropriate corrections for radioactive decay.

The standard was prepared by diluting a portion of the original 2 C ^{51}Cr solution to 40 Ci in 2 ml of distilled water (2.500 x 10^{-4}g of Cr). Knowing the original ration of chromium-51 to chromium in the leaf soaking solution, and making certain assumptions, the total chromium concentration in the crayfish was calculated. The assumptions are that both forms of chromium behave the same way in biological systems (that is, the original $^{51}Cr:Cr$ ratio will remain the same during all biological processes) and that there is no isotopic replacement of chromium-51 for chromium already present in the crayfish before the experiment began. The following equation was used to determine chromium concentration:

Cr concentration in sample:

$$\frac{\text{counts/min of sample}}{\text{counts/min of standard}} \times \frac{2.500 \times 10^{-4}\text{ g of Cr in standard}}{\text{sample wet weight (g)}}$$

The error introduced by dissimilar sample geometries[1] was reduced by immobilizing each crayfish by attaching it to a heavy posterboard card with rubber bands. The card was placed in a thin plexiglass box with the suture between the animal's thorax and abdomen clearly centered. The box could be placed inside the detector in a reproducible position. Water samples, leaf disc samples, and the standard samples were assayed in 25-ml scintillation vials taped into the center of the box.

The box was lined with a plastic bag that was discarded after each crayfish to prevent contamination. Each crayfish was wrapped in water-soaked cheesecloth to minimize dehydration and assayed for 0.5 h. This was not long enough to reduce analytical uncertainty to 1%[2] but was the maximum amount of time feasible without injuring the crayfish. Crayfish in the Cr and C groups were also attached to cards and wrapped in wet cheesecloth for 0.5 h every week; however, they were not taken to the building that housed the detector nor were they placed in the plexiglass box. Each Cr and C crayfish was assayed at least once during the experiment; none indicated any chromium-51 contamination. Additional monitoring included counting chromium-51 labeled leaf discs and water samples from various stages in the food preparation and feeding process to indicate final chromium content of discs and chromium loss due to leaching. The water in the two aquaria holding Cr* crayfish was assayed weekly and showed no contamination, supporting the assumption that the animals were received no chromium from the water.

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[1] Some error was introduced into the results because the crayfish varied in size and were of very different dimensions from the standard in a cylindrical vial. It is particularly important to center each sample in front of the detector and to maintain the same distance from sample to detector.

[2] Analytical uncertainty is expressed as $\frac{\sqrt{b}}{b}$, where b is the total number of gamma rays detected in the peak. It is an estimate of the precision of multiple analyses (Koons and Helmke 1978) and does not include error introduced by sample weighing and handling or biological variation.

Behavioral observations--Weekly behavioral observations alternated so that any one crayfish was observed only once in 2 weeks. The location, position with respect to shelter, activity, interaction with other crayfish, and dominance in the interaction were recorded. The 10 possible locations included nine squares on the bottom of the tank and the screen divider. Activities were classified as:

1. Non-interactive: walking, climbing (on glass sides, aeration tube, or screen), swimming, feeding, grooming, and motionless.
2. Interactive: touching, aggression, and retreating.

All patterns except motionless and retreating were "volitional," active behavior patterns not initiated by another animal. The ethogram was modified according to Stein and Magnuson (1976). Walking, climbing, swimming, motionless, grooming, aggression, and touching were as defined. Because there was no substrate in the tanks, probing, digging, and burying were not included; copulation did not occur between females; chelae (pincerlike claws) display in response to a predator was not possible; and feeding consisted of using the pereiopods to pick up feces and move it toward the mouth. Aggression described the dominant individual in an encounter; retreating indicated the subordinate.

Individual crayfish were observed at 10-sec intervals. Each of the three crayfish in the aquarium half was observed in turn, thus each was observed every 30 sec. A "set" consisted of 10 observations per crayfish. This procedure was then repeated at another aquarium. Each week, five sets of observations were made on each aquarium, two during light and three during darkness with 25-W red light bulbs placed in the sockets. Nail polish dots on the sides of the body and on the chelae permitted individual recognition at any angle. The order of observation of individuals in a tank, of tanks within a trough, and of troughs was randomized each week. Complete randomization of the sequence of individuals to be observed might have resulted in disturbing the crayfish by moving from tank to tank too frequently; therefore, all crayfish in a tank were observed at once.

Activity was higher and more varied at night, so only night-time observations were analyzed. The number of location changes observed for each crayfish during the three observation sets on one night was summed and a median found for all six crayfish of each treatment observed at night. This procedure was repeated for number of actions. The small number of events did not permit individual types of behavior.

Friedman's Randomized Blocks test was used to analyze the effects of treatment on activity (separately for location changes and actions). Medians were ranked regardless of treatment or date of observation and the rank compositions of the three crayfish groups were compared. The effect of time on behavior, regardless of treatment, was analyzed in the same way.

Termination of experiment--All crayfish were fed control food twice during the ninth week to clear their guts of unassimilated chromium. The Cr* crayfish were radioassayed again.

Mortalities were recorded and dead crayfish frozen individually in polyethylene bags. Mortalities among the groups were compared with the Mann-Whitney Wilcoxon Test. Crayfish were ranked by date of death, independent of treatment, and each pair of groups was compared. After the last assay, remaining crayfish were frozen and stored for tissue metal analysis as described for the caging experiment. To prevent interorgan metal diffusion they were not allowed to thaw until dissection (Luoma 1976). Three surviving crayfish from each treatment were later dissected.

Results--

Chromium uptake in chromium-51 labeled crayfish--Crayfish accumulated statistically significant amounts of chromium from their food during an 8-week period (Figure 24). The uptake was most rapid during the first week of feeding, increasing from 0 to 16 ppb, but concentration more than doubled in the next 7 weeks. At the end of the first week of feeding, crayfish had retained 2.75% of the chromium ingested during that week. After the eighth week, they had retained 1.72% of the chromium ingested during the experiment. The difference in chromium concentration after 1 week on uncontaminated food (week 9 to week 10) is 9.6 ppb, a reduction of 23.7%. The mean chromium concentration in Cr* crayfish was directly proportional to cumulative chromium ingested per crayfish (b = 0.0041 ***) (Figure 25).

There was no significant difference in total food consumption between the three groups of crayfish (Kruskal-Wallis Test, H = 0.6585 n.s., d.f. = 2). Mean total consumption for all crayfish for weeks 1 through 9 was 24.5 discs, the median was 19 discs, and the range 0 to 74 discs. Mean dry weight per disc was 1.40 mg and there was no difference in weight between control and chromium-contaminated discs (t-test, n = 23, t = 1.27 n.s.). Chromium-51 labeled discs had a mean chromium concentration of 263.4 ppm (range 118.7 to 523.0 ppm). There was a mean of 0.384 g chromium per disc.

Lethal and sublethal effects of chromium ingestion--Mortality rate did not differ among treatments (Table 23). Treatment groups did not differ in median number of actions (Figure 26) (Friedman's Randomized Blocks Test, $\chi^2 = 1.63$ n.s., d.f. = 2) or in median number of location changes ($\chi^2 = 1.63$ n.s., d.f. = 2). Activity appeared to decline over time for all treatments (Figure 26), but differences were not significant between observation dates either for number of actions (Freidman's Randomized Blocks Test, $\chi^2 = 4.40$ n.s., d.f. = 3) or for number of location changes ($\chi^2 = 5.80$ n.s., d.f. = 3).

Further attempts to determine the cause of the activity decline were not helpful. There was no significant correlation between total food consumption and number of actions (r = 0.07 n.s.) or number of location changes (r = 0.28 n.s.). The correlations between final chromium concentration (for Cr* crayfish only) and activity also were not significant (r = -0.08 n.s., for number of actions; r = -0.04 n.s., for number of location changes).

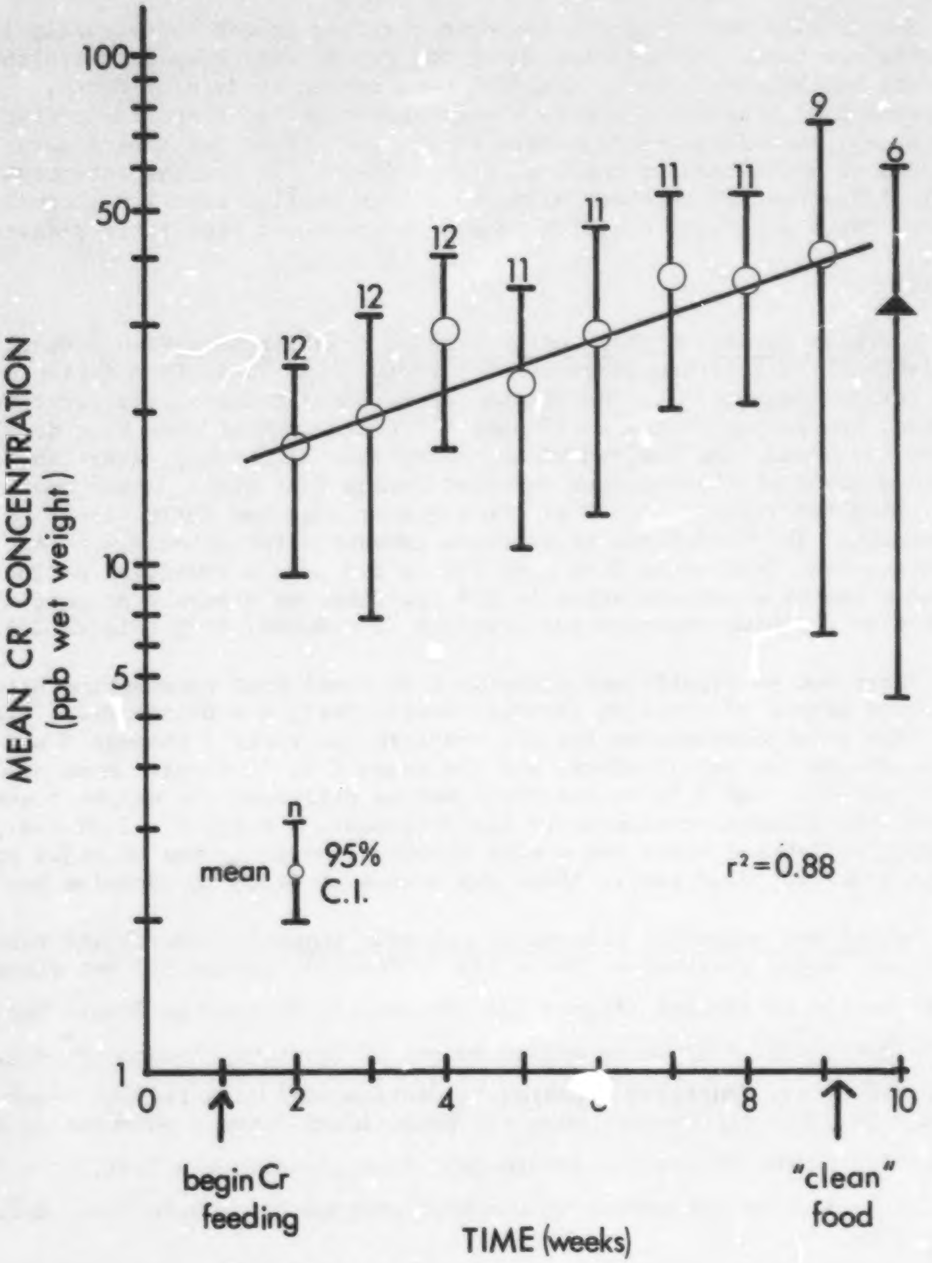

Figure 24. Mean chromium concentration over time in chromium-51 labeled crayfish. Crayfish were fed food without chromium after week 9. The regression equation for weeks 1 through 9 is: log Cr concentration = $-7.86 + 0.054$ (time). The regression coefficient is highly significant ($P < 0.001$). N - sample size and C.I. = confidence interval.

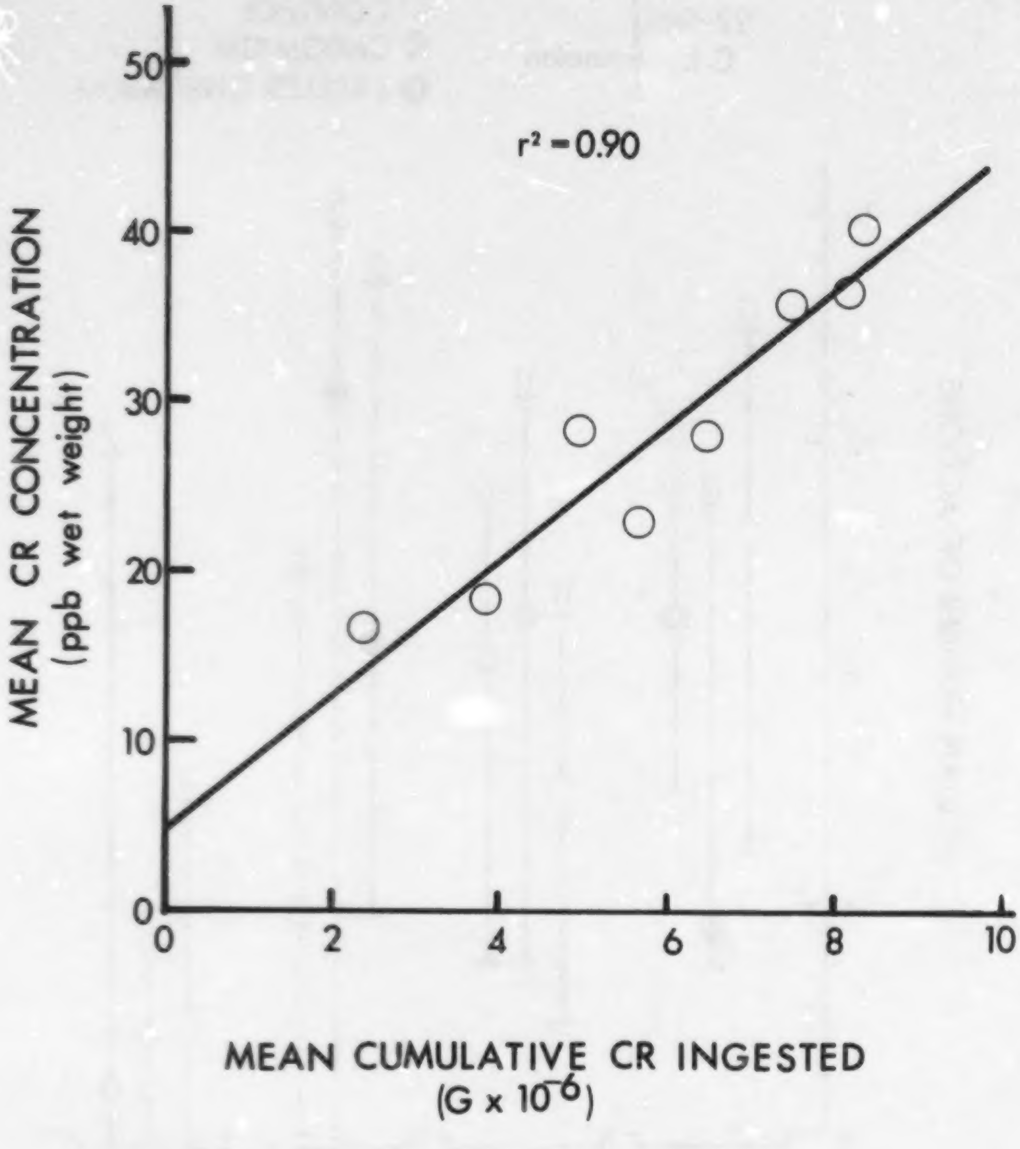

Figure 25. Relationship between whole-body chromium concentration and total chromium ingested by chromium-51 labeled crayfish. Each point represents the mean for all crayfish for 1 week in the experiment. The regression equation is: Cr concentration - 0.0041 (Cr ingested) + 4.9. The regression coefficient is highly significant ($p < 0.001$).

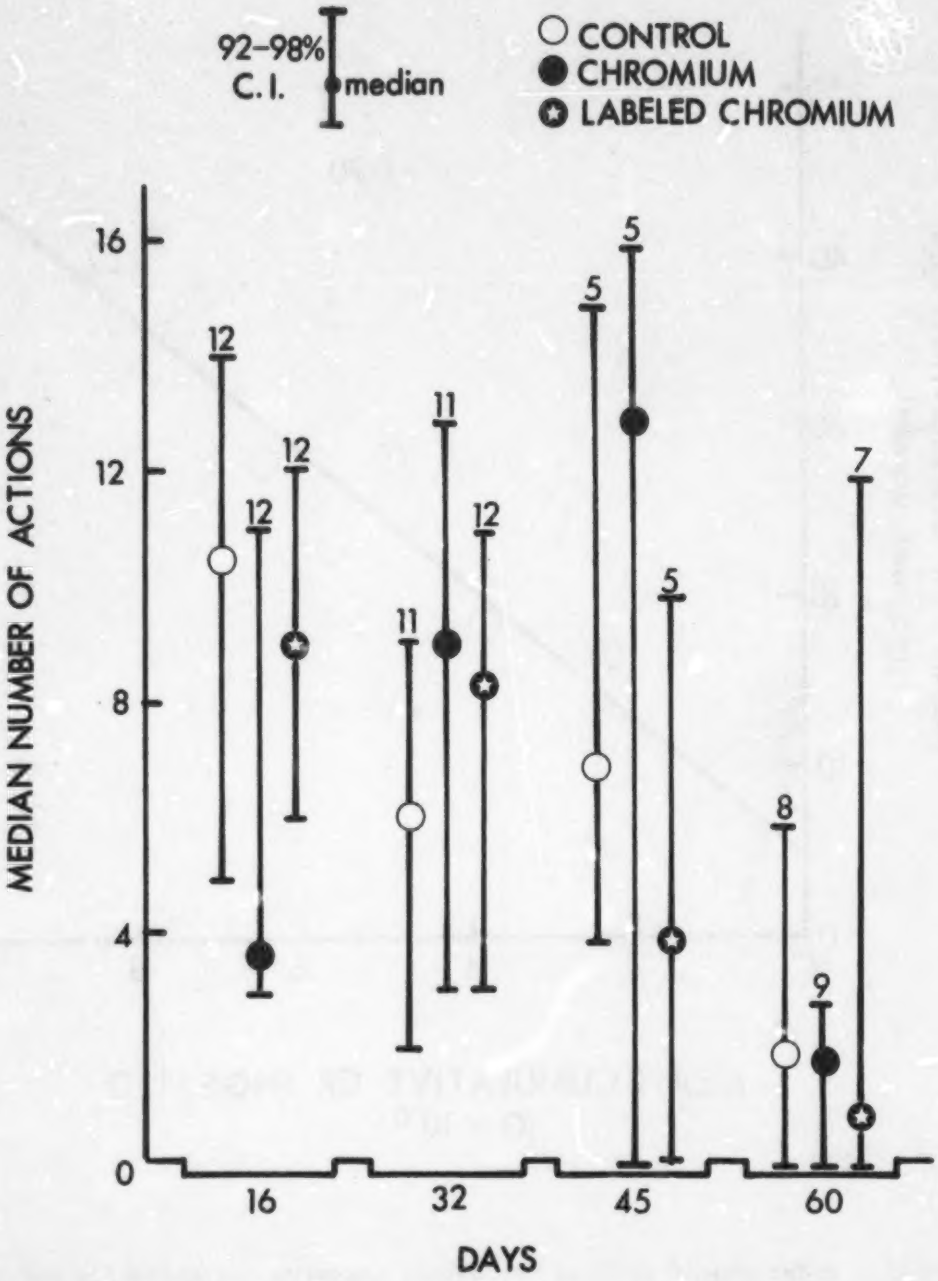

Figure 26. Median number of actions performed by the three groups of crayfish on four dates during the experiment. Confidence intervals of 92 to 98% and sample size are given; the 95% confidence intervals did not correspond exactly to an integral number of actions.

TABLE 23. LETHAL EFFECTS OF CHROMIUM INGESTION

Mortality	Treatment[+]		
	C	Cr	Cr*
No. of crayfish surviving experiment (out of initial 12)	7	9	6
% survival	58	75	50

Mann Whitney Wilcoxon test

Treatment pair tested[+]	Significance level[‡] of differences between treatments ( )	
Control vs. Cr	92.9 - 87.5	n.s.
Chromium vs. Cr*	91.7 - 86.5	n.s.
Control vs. Cr*	< 46.5	n.s.

[+] C = control, Cr = fed chromium, Cr* = fed chromium labeled with ^{51}Cr.

[‡] Exact significance could not be found because of the discrete nature of the data.

Metal uptake--Chromium concentrations were highest in the hepatopancreas for two crayfish groups (Table 24), but there was no significant difference in concentration between treatments (Kruskal-Wallis Test, H = 2.489 n.s.). Since the crayfish were not differentially exposed to any other metals, and since visual examination gave no evidence that treatment group affected concentration of these metals (Harrell 1978), treatments were pooled for metals other than chromium. Barium occurred in the heptopancreas and exoskeleton. Iron and selenium appeared only in the hepatopancreas. Zinc was most concentrated in muscle and hepatopancreas, but also was found in the exoskeleton.

Although cadmium occurred in the leaf discs (Table 25), it was not present at detectable levels in any crayfish tissues. The value of 200 ppm Cr for Cr* discs obtained by the University of Wisconsin Nuclear Reactor analysis was lower than the 263.4 ppm obtained by the chromium-51 labeling and radioassay.This may be a result of a wide variation among samples or the difference in analytical method. The values for all other metals in C and Cr discs were similar, as expected.

TABLE 24. METAL CONCENTRATION IN TISSUES OF CRAYFISH FED CHROMIUM IN THE LABORATORY (MEAN ± S.E., NO. OF SAMPLES)[+]

Metal	Crayfish group	Tissue concentration (ppm dry weight) Hepatopancreas	Muscle	Exoskeleton
Cr	C	3.153 ± 0.5108 (3)	4.076 ± 1.698 (3)	--- (3)
	Cr	2.297 ± 0.3730 (3)	--- (3)	1.018 ± 0.8315 (3)
	Cr*	5.943 ± 2.333 (3)	1.886 ± 1.540 (3)	--- (3)
Ba	all	24.03 ± 4.43 (9)	--- (9)	183.3 ± 27.0 (9)
Cd	all	--- (9)	--- (9)	--- (9)
Fe	all	563.0 ± 125.9 (9)	--- (9)	[‡] (9)
Se	all	4.383 ± 0.232 (9)	--- (9)	--- (9)
Zn	all	1,250.0 ± 305 (9)	2,108 ± 278 (9)	476 ± 45 (9)

[+] Analysis is broken down into the three crayfish groups for Cr concentrations only, since crayfish were differentially exposed only to chromium. --- = below detection limit.

[‡] Only one sample was above detection limit.

Discussion--

Chromium uptake--Crayfish can assimilate chromium incorporated in their food supply, although the amount of uptake from ingestion is low. An assimilation of less than 3% of the amount ingested agrees with the findings reported in the literature. Rats that had not been fed absorbed 6% of the chromium they ingested, while rats that had been fed absorbed only 3% (Mackenzie et al. 1959). Rainbow trout (*Salmo gairdneri*) assimilated no hexavalent chromium even when it was placed directly in the digestive tract.

Despite this low level of uptake, ingestion may be an extremely important mode of uptake where chromium concentrations are high in particulate matter and low in dissolved form. This is the situation in the ash effluent drainage system of the Columbia Generating Station. Chromium concentrations in suspended particulate matter from the ashpit drain were over 1,000 ppm shortly after the generating station began operating (Helmke et al. 1976a). In contrast, dissolved chromium in the ashpit drain ranged from 0.006 to 0.028 mg/liter from November 1976 to April 1977 (Andren et al. 1977). Organisms collected from the ashpit drain had elevated concentrations of chromium and barium (Schoenfield 1978) and crayfish caged at effluent-affected sites accumulated chromium and other metals. Since

TABLE 25. METAL CONCENTRATIONS IN LEAF DISCS FED TO CONTROL (C) AND CHROMIUM-FED (Cr) CRAYFISH[+]

Metal	Concentration (ppm dry weight) C discs	Concentration (ppm dry weight) Cr discs
Ba	---	---
Cd	46.02	30.08
Cr	3.265	200.7
Fe	513.5	328.2
Se	---	---
Zn	1,223	1,065

[+] Only one sample of each food was analyzed. --- = below detection limit.

dissolved chromium remains low, uptake from ingested chromium may be far more important in the ash basin drainage system than laboratory experiments predict.

The rate of chromium uptake during the experiment appeared linear. There was no indication that chromium concentration began to level off during the experiment. A leveling off or distinct reduction in the rate of increase would indicate that a stable body burden of chromium had been reached, with the organism in a state of equilibrium with its environment or food. Maximum chromium uptake by trout was reached after 10 days in solutions of low chromium concentration (0.0013 and 0.01 mg Cr/liter), but in more concentrated solutions (0.05, 0.1, and 0.15 mg Cr/liter), there was no sign of leveling off after 30 days of uptake (Fromm and Stokes 1962). Since uptake mechanisms from food and water are different (from digestive tract as opposed to gills and/or integument), a direct comparison with the trout data should not be made. However, a failure to reach equilibrium after more than 40 days of exposure to dietary chromium at concentrations > 200 ppm does not seem inconsistent, especially since the percent assimilation was so low.

Whole-body chromium concentrations, after exposure to uncontaminated food (week 10), were 24% less than the previous week. This may indicate that unassimilated chromium present in the gut during radioassay in week 9, and presumably all previous weeks, was egested by week 10. It may also be due to loss of assimilated chromium from the tissues during the previous week. Both egestion and tissue elimination probably were operating, but the relative contribution of each to the decline in chromium concentration can not be ascertained from the data. Unassimilated chromium in the gut apparently does not contribute significantly to whole-body concentrations. Elwood et al. (1976) found that chromium concentration in *Tipula* did not decrease after gut evacuation. Schoenfield (1978) presents corroborating evidence. Gut evacuation did not appreciably reduce the whole-body chromium concentration of *Asellus racovitzai* collected from sites high in suspended particulate and soluble chromium concentrations. Apparently, chromium was either assimilated readily from the digestive tract or egested rapidly and little remained in the gut contents to affect the analysis. The crayfish were radioassayed within a few hours of feeding; thus, the undigested food probably remained in the stomach containing chromium that the animals had no opportunity to assimilate. However, if the results obtained by Elwood et al. (1976) and Schoenfield (1978) can be applied to crayfish, this undigested chromium in week 9 was digested by week 10 and played no part in the decline in chromium concentration. The assimilation ratio of less than 3% may indicate that most chromium was egested quickly or that it was assimilated and excreted rapidly. The latter explanation is more consistent with the results obtained by Elwood et al. (1976) and Schoenfield (1978). For these reasons it is suggested that chromium reduction in the crayfish is attributable primarily to tissue loss rather than to egestion of unassimilated chromium.

Lethal and sublethal response--There is no evidence that chromium ingestion caused crayfish deaths or behavioral differences. However, small sample sizes and wide variability between individual crayfish might have masked considerable behavioral differences. The high overall death rate and the apparent decline in activity as the experiment progressed were probably due to poor health and low food consumption. The maximum number of discs consumed by any crayfish was 74, with a corresponding total dry weight of 0.104 g in 9 weeks or less than 5% of the wet weight of a 3-g crayfish. This is not an adequate ration. Food consumption in most animals declined as the experiment progressed.

The lack of natural wintertime temperatures and photoperiods during the previous winter in the laboratory probably caused the poor health and low appetite of the crayfish. The animals did not experience the 4°C water and long dark period required for proper ovarian development (Aiken 1969a). Aiken (1969b) also reports high molt mortality among crayfish kept on an abnormal photoperiod schedule. Other physiological processes such as metal assimilation and transport may have been impaired in the experimental crayfish. Therefore, the tissue metal locations and concentrations may not be the same as those expected in healthy crayfish with adequate food consumption.

Tissue metal uptake--The lack of statistical difference between crayfish groups in hepatopancreas chromium concentration was unexpected in view of the increased whole-body concentration in the Cr* crayfish.[3] Small sample size could be the sole reason for this result, but it is more likely that the mean whole-body increase of 0.196 ppm (dry weight) provided such a small additional amount of chromium to the hepatopancreas relative to the pre-experimental level that no increase was detectable. It is also possible that isotopic exchange was replacing the chromium already in the crayfish with the labeled chromium, thus, there would be no change in total chromium concentration.

Mean chromium concentrations obtained by neutron activation analysis for three of the Cr* crayfish were 1.9 ppm for muscle, below detection limit for exoskeleton, and 5.9 ppm for hepatopancreas. These differences in tissue concentrations indicate that the hepatopancreas, and to a lesser extent the muscle, were sites of chromium concentration within the body.

Evidence from the literature supports these results. Schiffman and Fromm (1959) found that exposing rainbow trout to chromium in their water resulted in small amounts of chromium in the muscle and significant amounts in the spleen, gall bladder and bile, kidney, and liver. Of all tissues studied (blood, spleen, liver, muscle, gut, pyloric caeca, stomach, and kidney) in rainbow trout, only the muscle and blood failed to accumulate chromium at concentrations higher than those in the water (Knoll and Fromm 1960). Crustacean hepatopancreas and vertebrate liver perform similar physiologic functions, thus, it is useful to compare metal concentrations in the two tissues. In the lobster, *Homarus americanus*, chromium is highest in the gills (the site of absorption from water) and lowest in the exoskeleton; the hepatopancreas is an important storage site for chromium and other metals (Van Olst et al. 1976). After exposing the crab, *Podophthalmus vigil*, to chromium in the water, Sather (1967) detected the following decreasing order of radioactivity in the tissues: gills > muscle > midgut gland (hepatopancreas) > carapace > blood. Blood had low chromium levels in all of these studies, leading several authors to conclude that blood is the main chromium transport mechanism and that it loses its chromium rapidly to other tissues. Thecarapace was metabolically inactive in the lobster and crab studies, which explains the low or undetectable exoskeleton chromium levels in the crayfish.

Conclusions--

Crayfish assimilate ingested chromium, although the percent assimilation and total amount are small. The relative importance of the food and water pathways was not determined, but based on its chemical properties, dietary uptake appears to be more important for trivalent chromium (see literature review, Appendix D). Based on studies by Schroeder (1973), chromium in the leaf discs was probably in trivalent form, as is the chromium in the sediments, detritus, and organisms of the ashpit drain system.

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[3] Because neutron activation analysis of the whole body burden was not possible no direct comparison of the two assay methods can be made.

The chromium uptake by laboratory crayfish was not high enough to cause significant mortality or behavior changes. However, under more normal food consumption patterns by healthy crayfish, the 200 ppm of chromium in the food might have had detectable effects. Less tolerant life stages or organisms may have been significantly affected. Although the laboratory-exposed crayfish appeared unaffected by chromium in their food and assimilated very little, this experiment probably underestimates the magnitude of the effects in the ashpit drainage system where particulate chromium concentrations are elevated over background levels by several orders of magnitude.

Long-Term Exposures of *Asellus racovitzai* to Ash Effluent in the Laboratory

Introduction--

The purpose of this study was to investigate whether *Asellus* exposed to water and food from the ashpit drain prior to the spring reproductive pulse would grow more slowly and produce fewer young than *Asellus* given water and food from the mint drain. Whether any observed differences in growth or fecundity were caused by the water and food were also to be determined; thus, *Asellus* were fed ashpit drain food in control water and control food in ashpit drain water.

By hand-netting, it was discovered that *Asellus* were less abundant in the ashpit drain (sites A2, A3, and A4) than in the mint drain (site A1) and that few of them colonized the artificial substrates in the ashpit drain. The reason for this did not appear to be acute toxicity because the crayfish studied earlier survived as well in the ashpit drain as in the mint drain. Instead, the lower metabolic rates of the caged crayfish in the ashpit drain suggested that sublethal effects on growth and reproductive success decreased *Asellus* number.

Asellus racovitzai begin to mate in February in central Wisconsin (Herbst 1975). Young are released from the females starting in April and May. Several fast-growing summer generations follow and over-wintering individuals are born in late summer or early fall. Winter-generation adult isopods are larger and bear more young per female (Seidenberg 1969).

Materials and Methods--

The experimental design was as follows: Treatments A (control food and water) and D (ashpit drain and water) were expected to differ the most; treatments B (ashpit food and mint drain water) and C (ashpit water and mint drain food) were designed to separate the effects of food and water. Another concern was that the *Asellus* in treatment C might ingest the precipitated chemical floc in the ash effluent in addition to feeding on the uncontaminated leaf discs. Therefore, a sub-experiment was added to compare treatment C to a fifth treatment, E, This treatment was identical to C, but the ashpit drain water was filtered (0.45 Millipore) to remove particulates.

The experimental apparatus was designed to hold animals in clear PVC tubes (8.2 cm high x 5.8 cm diameter) in 500-ml pyrex beakers. The bottom of the tubes consisted of PVC-coated fiberglass window screen. The animals could be photographed for growth measurements in the tubes and water could be changed without handling the animals. The beakers were suspended in a water bath. Temperature in the bath was controlled by circulating water in copper tubing between a Frigid Units chiller and the water bath outside the beakers.

Specimens of *Asellus racovitzai* were captured by hand net in the mint drain. Water was collected in 19-liter (5 gal) polyethylene containers at the mint drain site, A1, and ashpit drain site, A4 (Figure 1). Leaves were soaked in ashpit drain (A4) or mint drain (A1) water for 2 weeks prior to feeding them to *Asellus*. Leaves were collected from a sugar maple, *Acer saccharum*, in the fall, oven dried, and stored in plastic bags. Individual leaves were placed in separate compartments in nylon bags; the bags were suspended with bricks and floats in the field at sites A1 and A4 for 2 weeks. Placement of bags in the field was staggered so that a set soaked for 2 weeks was ready to be used each week. A 15-mm diameter stainless steel cork borer was used to cut discs from the soaked leaves.

A preliminary experiment indicated that *Asellus* could be transferred directly to ashpit drain water from mint drain water without mortalities (Table 26) and that the isopods did eat the leaf discs.

Five individuals (64 to 96 mm long, $x = 80$) were placed in each of 70 beakers on 17 Dec. 1977. Each beaker contained mint drain water and leaf discs. Animals were checked and the dead replaced daily. Exposure to treatment water and leaf discs began on 20 Dec. 1977. Fourteen beakers were randomly selected for each treatment. Dead animals were no longer replaced. Weekly support of the experiment proceeded as follows:

Day 1 - Collect water and leaf bags from mint drain and ashpit drain. Place new leaf bags into mint drain and ashpit drain for 2-week incubation. Cut leaf discs; hold water and discs in water bath.

Day 2 - Change water and food in each beaker; remove dead animals. Water chemistry.

Day 3 - Re-randomize the beakers.

Day 4 - Photograph animals (every 2 to 3 weeks) for growth.

Day 5 - Change water and food in each beaker; remove dead animals. Water chemistry.

Days 1 through 7 - Check temperatures, pump, etc.

The water bath was held at 3.81 ± 0.23°C and the soft-on and soft-off light controls were set at 9.5 h light:14 h darkness for the first 6 weeks. Temperature and photoperiod were then accelerated weekly to simulate mid-April conditions by late February, thereby encouraging earlier reproduction.

Temperature and Photoperiod Schedule

Dates	°C	Lights On	Lights Off
20 December - 29 January	3.81 ± 0.23	7:00	16:30
30 January - 5 February	5.92 ± 0.88	6:45	16:54
6 February - 12 February	7.75 ± 0.21	6:24	17:18
13 February - 19 February	10.01 ± 0.07	6:06	17:42
20 February - 26 February	11.82 ± 0.04	5:48	18:00
27 February - 21 March	13.92 ± 0.04	5:30	18:24

Results and Discussion--

Survival of *Asellus* among the four treatments (A through D) in the main experiment was the same (Figure 27). Shed exoskeletons (indicating growth) were sometimes found in the first weeks of the experiment (20 December to 24 January) and some mating was observed in late January and February. However, the entire group stopped molting in late January and many animals began dying in all treatments in late February. None of the females showed evidence of carrying young and the experiment was terminated; unfortunately no growth or fecundity data was obtained. The reason for these mortalities in a usually hardy animal was explored and it was learned that other researchers had experienced a similar mid to late winter loss of isopods and amphipods in the laboratory (Herbst, personal communication and Kitchell, personal communication) and in the field (Herbst 1975). It was decided to wait for the hardier summer generations before continuing.

TABLE 26. PRELIMINARY EXPOSURE OF *ASELLUS RACOVITZAI* TO MIXTURES OF ASHPIT DRAIN (A3) AND CONTROL (A1) WATER

Day	% alive 0:100	25:75	50:50	100:0[+]
1	97	100	97	100
2	97	100	97	100
5	97	100	97	100
8	97	100	97	100
No. of isopods	32	32	32	32

[+] %ashpit drain: % control water.

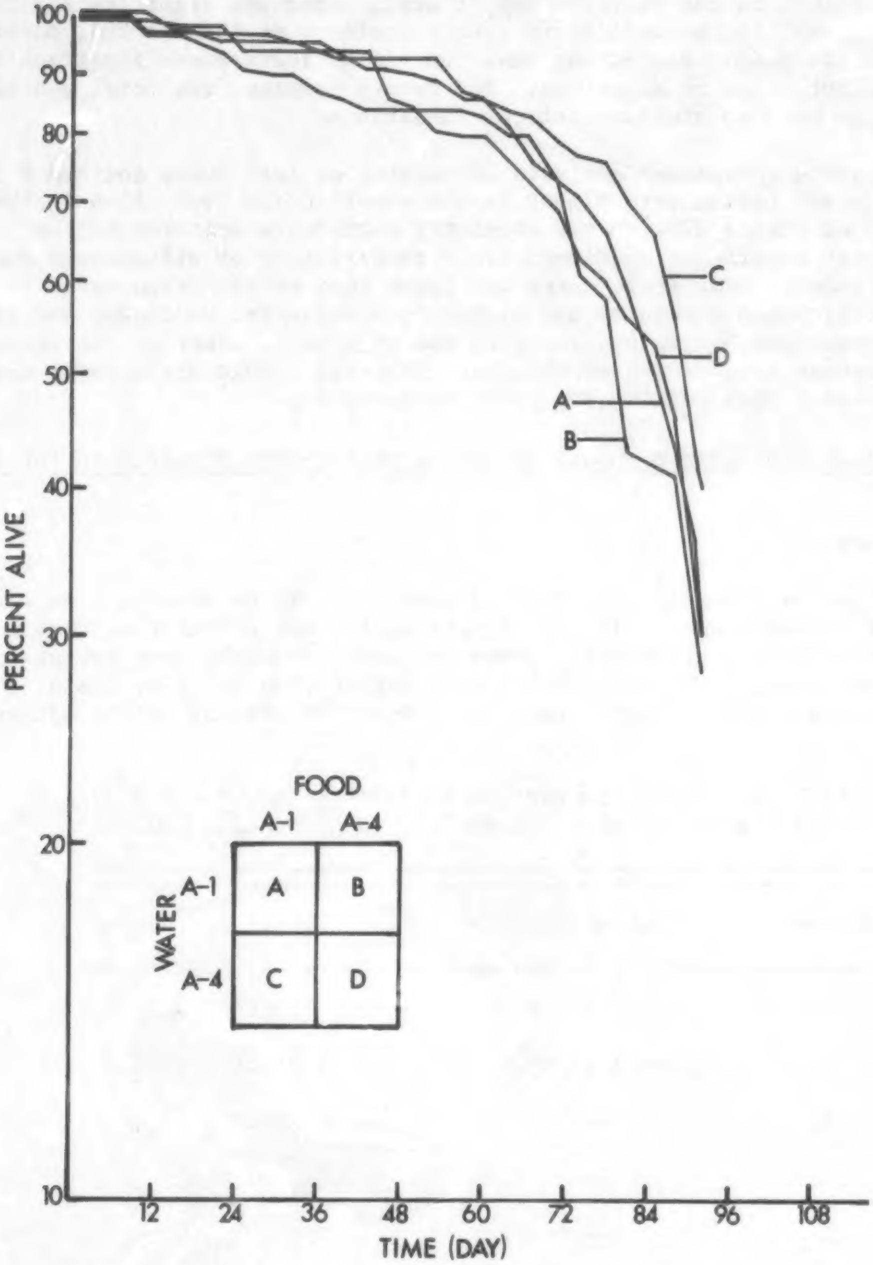

Figure 27. Survival of *Asellus racovitzai* exposed to leaf litter and water from locations upstream (sampling station A1) and downstream (station A4) from the ash effluent. Differences between treatments were not significant ($p < 0.001$) using a Mantel-Haenszael test that computes χ^2 values for observed mortalities during each time period.

Survival in the filtered ashpit drain water was significantly lower than survival in the unfiltered ashpit drain water (Figure 28), possibly because the 0.45- filter may have removed microorganisms important to *Asellus* nutrition or digestion. For future studies, the total ash effluent should be used to simulate natural conditions.

Neutron activation analysis of samples of leaf discs confirmed that chromium and barium were higher in the ashpit drain food than in the mint drain food (Table 27). Water chemistry parameters measured in the laboratory experiment confirmed field observations of differences due to the ash effluent. Mint drain water was lower than ashpit drain water in conductivity and turbidity and higher in alkalinity, hardness, and pH (Table 28). Dissolved oxygen was lower in the mint drain water at the warmer temperatures late in the experiment. Filtered ashpit drain water was lower in turbidity than unfiltered ashpit drain water.

Short-Term Exposures of Adult and Young-of-the-Year *Gammarus* to the Ash Effluent

Introduction--

Time did not allow the *Asellus* experiment to be repeated, so some simpler procedures to test the effects of the ash effluent on a more sensitive benthic crustacean, *Gammarus pseudolimnaeus*, were designed. Since *Gammarus* is common to Rocky Run Creek, rather than the mint drain, the results would tell us more about the downstream effects of the effluent. In

TABLE 27. TRACE ELEMENT CONCENTRATIONS (PPM ± 1 S.D.)
IN LEAVES PREPARED FOR FEEDING *ASELLUS* MARCH 1978

Element	Control (A1)	Ashpit drain (A4)
Cr	7.61 ± 3.87	13.76 ± 2.77
Zn	1,199.1 ± 210.3	1,224.0 ± 248.6
Ba	--[+]	314.8 ± 79.1
Cd	--	--
Se	--	--
Sb	--	--
Fe	2,988.7 ± 933.3	1,510.0 ± 454.0

[+]-- = below detectable limits.

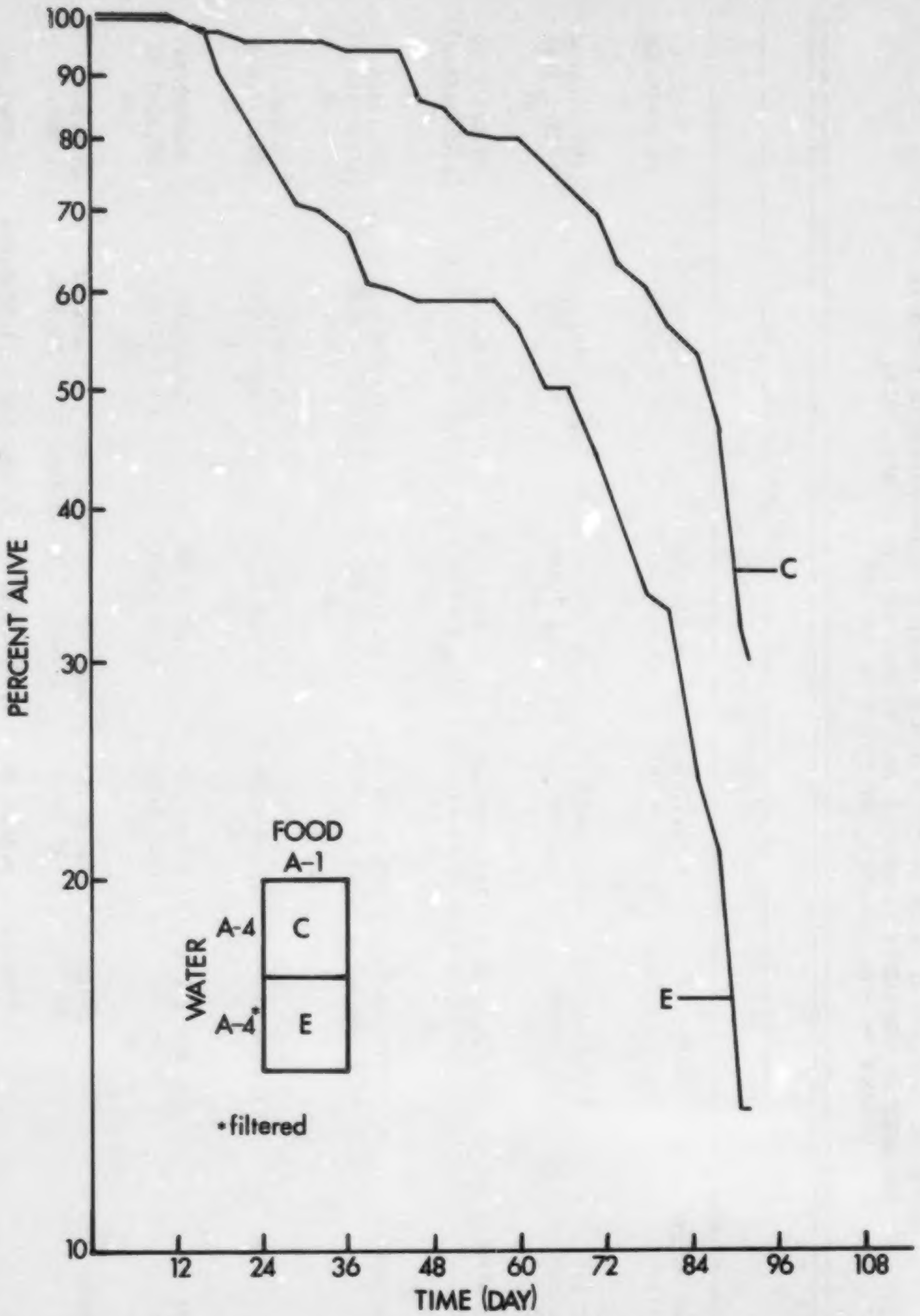

Figure 28. Survival of *Asellus racovitzai* exposed to filtered (sampling station E) and unfiltered (station C) ashpit drain water. (Differences were statistically significant, $p < 0.001$, Mantel-Haenszael test (Snedacor and Cochran 1967).

TABLE 28. SUMMARY OF WATER IN PHYSICAL AND CHEMICAL MEASUREMENTS OF WATER IN THE *ASELLUS* LABORATORY EXPERIMENT FROM 23 DEC. 1977 TO 17 MAR. 1978. AVERAGE, ± S.D.; (RANGE); AND NUMBER OF SAMPLES

	Treatment				
	A	B	C	D	E
Dissolved oxygen (mg/liter)	8.60±1.48 (6.16-10.26) 6	8.78±1.47 (6.17-9.96) 6	9.62±0.68 (8.91-10.75) 6	9.71±0.60 (9.09-10.76) 6	9.54±0.76 (8.80-10.68) 6
Conductivity (mhos/cm) at 25°C	474.48±47.02 (425-636) 23	471.61±37.23 (426-553) 23	1,167.52±109.02 (932-1,294) 23	1,154.48±116.14 (943-1,290) 23	1,160.65±119.50 (905-1,316) 23
Total Alkalinity (ppm)	210.77±17.91 (164.38-243.84) 20	210.59±16.15 (171.44-240.16) 20	95.01±15.24 (65.18-125.94) 20	94.84±16.12 (163.24-127.09) 20	91.62±16.69 (62.08-125.37) 20
Hardness (ppm)	250.79±16.53 (232-291.88) 20	249.04±16.52 (231.6-291.89) 20	133.91±23.18 (99.20-173.6) 20	133.07±22.87 (97.20-162.80) 20	135.19±26.86 (97.20-195.62) 20
pH	7.83±0.11 (7.58-7.95) 19	7.83±0.10 (7.68-8.08) 19	7.47±0.31 (7.28-7.74) 18	7.44±0.11 (7.30-7.68) 19	7.46±0.11 (7.3-7.66) 18
Turbidity	1.35±0.41 (0.8-2.5) 21	1.28±0.51 (0.7-3.4) 21	5.62±6.90 (2.2-35.0) 21	5.45±6.90 (1.8-32.0) 21	0.60±0.53 (0.25-2.0) 21

Temperature Date	23 Dec- 30 Jan.	Week of 30 Jan.	Week of 6 Feb.	Week of 13 Feb.	Week of 20 Feb.	28 Feb.- 21 Mar.
°C	3.81±0.23 167	5.92±0.88 48	7.75±0.206 48	10.01±0.068 48	11.82±0.036 48	13.97±0.042 72

Experiment I, it was hyposethsized that the younger animals would be more sensitive than adults. First, the toxicity of the effluent to young-of-the-year was demonstrated, then a series of dilutions of ash effluent in Rocky Run Creek water was selected to determine a concentration not acutely toxic but which might later be used to study growth and fecundity.

Materials and Methods--

Experiment I--*Gammarus* from Rocky Run Creek above the ash effluent were collected on 31 May 1978 and placed in individual beakers in the same apparatus used for *Asellus* at 17°C. Aerators were added to the apparatus for this experiment. Twenty young-of-the-year (4.6 ± 0.6 mm body length) and 20 adult (9.8 ± 2.2 mm) amphipods were used. After 24 h acclimation in Rocky Run Creek water, the water was changed in all beakers with half the adults and half the young receiving ashpit drain water instead of Rocky Run Creek water. Treatments were randomly assigned to the beakers. The beakers were checked daily for 4 days. Dead amphipods were removed and preserved in 70% alcohol.

Experiment II--Young-of-the-year *Gammarus* were collected from Rocky Run Creek above the ash effluent on 28 June 1978 and placed individually in 60 beakers in the water bath at 17°C. After 24 h acclimation in Rocky Run Creek water, the water in all beakers was changed with groups of 12 randomly selected beakers receiving one of the following water types:

100% Rocky Run Water
75% Rocky Run + 25% Ashpit Drain Water
50% Rocky Run + 50% Ashpit Drain Water
25% Rocky Run + 75% Ashpit Drain Water
0% Rocky Run + 100% Ashpit Drain Water

Beakers were checked daily for dead animals for 4 days. Water was changed once at 48 h. Since no mortalities occurred and the ash effluent was half as concentrated as it had been in Experiment I, the experiment was continued for 4 more days with a water change at 48 h. The experiment was then terminated because heavy rains continued to dilute the effluent below the toxic level determined in Experiment I.

Results and Discussion--

Young instars of *Gammarus pseudolimnaeus* were more sensitive to the ash effluent than were large individuals of the same species (Figure 29). When the experiments were replicated on young instars using dilutions of the ash effluent to select a non-lethal concentration, heavy rains diluted the effluent below its toxic level (Figure 29). The threshold for acute toxicity of the ash effluent to young-of-the-year *Gammarus* falls between effluent concentrations of 1,100 and 1,900 μmhos as estimated by conductivity.

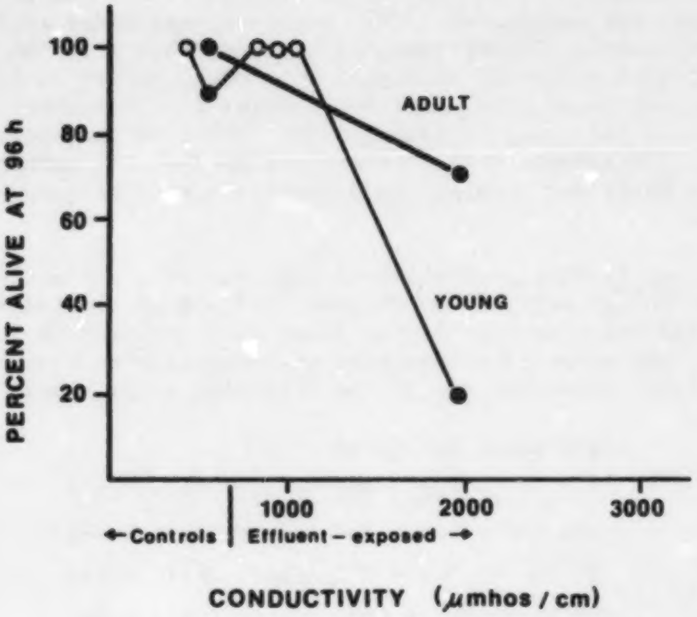

Figure 29. Percent survival of young and adult *Gammarus pseudolimneaus* exposed to the ash effluent for 96 h.

Spatial and Temporal Variability in Life Histories of Heptageniidae (Ephemeroptera)

Introduction--

Studies of the life cycles of Heptageniidae (Ephemeroptera) from various regions of North America have shown that, for many species, certain characteristics of the life cycle (such as timing and duration of emergence and hatching or number of generations per year) vary spatially and temporally. This study describes the life histories of seven species of Heptageniidae collected from the Wisconsin River and Rocky Run Creek over 3 years and compares them to life histories reported in the literature. These comparisons reveal patterns in the variability of life-history strategies and give insight into factors that may influence life cycles.

Study Area--

Heptageniidae were collected (with other aquatic invertebrates) during 6 months of the ice-free season from two sites on the Wisconsin River and two on Rocky Run Creek. Throughout the study area, the Wisconsin River has a sandy bottom with scattered, submerged logs and fallen trees. It is characterized by extensive seasonal floods. In 1974, water levels were high but steady after spring floods; they fluctuated widely in 1975, and were low but steady in 1976.

The upstream site in Rocky Run Creek had a substratum of muck, detritus, and patches of water star grass (*Heteranthera dubia*). The downstream station in Rocky Run Creek, near the mouth, had a sand bottom with detritus; *Potamogeton* sp. and *Ceratophyllum* sp. were extremely abundant. During annual spring floods, water from the Wisconsin River mixed with water from Rocky Run Creek at the downstream sampling site.

Dissolved oxygen values ranged from a low in both streams of 7.5 mg/liter on 30 Aug. 1974, to a high of 12.8 mg/liter and 13.3 mg/liter in the Wisconsin River and Rocky Run Creek, respectively, on 26 Oct. 1976. Temperatures in the river ranged from 5.6°C on 26 Oct. 1976 to 26.0°C on 7 Aug. 1975. In the creek the low was 7.3°C on 26 Oct. 1976 and the high was 26.5°C on 7 July 1976. In the Wisconsin River, average midsummer values for conductivity, total alkalinity, and hardness were 196 mhos/cm, 86, and 132 ppm, respectively. Average values of these parameters in Rocky Run were slightly more than twice as high. The pH of both streams averaged 7.8.

Materials and Methods--

Samples and physical measurements were taken during the ice-free seasons following the spring floods in 1974, 1975, and 1976. Four sites were sampled in 1974 and 1975; sampling was discontinued at the upstream Wisconsin River station in 1976.

Organisms were collected with basket-type artificial substrate samplers (Mason et al. 1970). These consisted of 20- x 29-cm chicken barbeque baskets (or similar wire replicas) filled with 4.5 kg of limestone gravel.

The samplers were suspended from overhanging branches 5 to 10 cm above the substrate. Three samplers were placed at each station. At monthly intervals, organisms were removed by shaking the samplers inside an aquatic D-frame net (1-mm mesh).

In 1976, additional samples were collected from a natural substrate at the downstream Wisconsin River station. Samplers were constructed from similar sections (21 x 15 cm) of a silver maple log (*Acer saccharinum*) from the Wisconsin River flood plain. The logs were weighted and suspended in a manner similar to the artificial basket samplers. There were two sets of three replicates. Every 2 weeks alternate sets were emptied, resulting in monthly samples from each set. The logs were drawn to the surface inside an aquatic net. After organisms were removed with a forceps, the logs were replaced in the water.

All samples were preserved in 70% alcohol in the field. Invertebrates were sorted, identified, and counted in the laboratory. Head capsule widths of heptageniid nymphs were measured to the nearest 0.25 mm with an ocular micrometer. Monthly size-frequency histograms were used to approximate times of emergence, hatching, and growth. Data from upstream and downstream sites in the same stream and from artifical and log samplers on the same dates were combined. There were no differences in life history interpretations between these locations and substrates.

Results--

Life histories of seven species of Heptageniidae are presented below in order of increasing number of generations per year (in the study area). Since no adult collections were made, times of emergence were determined approximately by the presence of large nymphs and the subsequent appearance of small nymphs.

Stenonema fuscom (Clemens) occurred only at the upstream site in Rocky Run Creek and had one generation per year (Figure 30a). Emergence occurred in late May to early June and young nymphs hatched about 1 month later. Growth was rapid in summer and by late fall the nymphs were almost full size.

The low numbers of *S. integrum* (McDunnough) from the Wisconsin River made interpretation and comparison of life-history patterns difficult. The smallest nymphs were found only in June, indicating that hatching occurred in spring. Also at that time, there was usually a range of size classes from small to medium (0.5 to 2.5 mm head-capsule width). After July, occasional individuals were caught through September. *S. integrum* probably has one generation per year.

S. exiguum (Traver) had two generations each year in the Wisconsin River (Figure 30b). One grew quickly during the summer and emerged in August and September. A longer winter generation followed, growing during the fall and emerging in the spring. Because times of emergence and hatching are not known exactly, the two generations could be either multiple cohorts or truly bivoltine (eggs of one generation are laid by adults of the

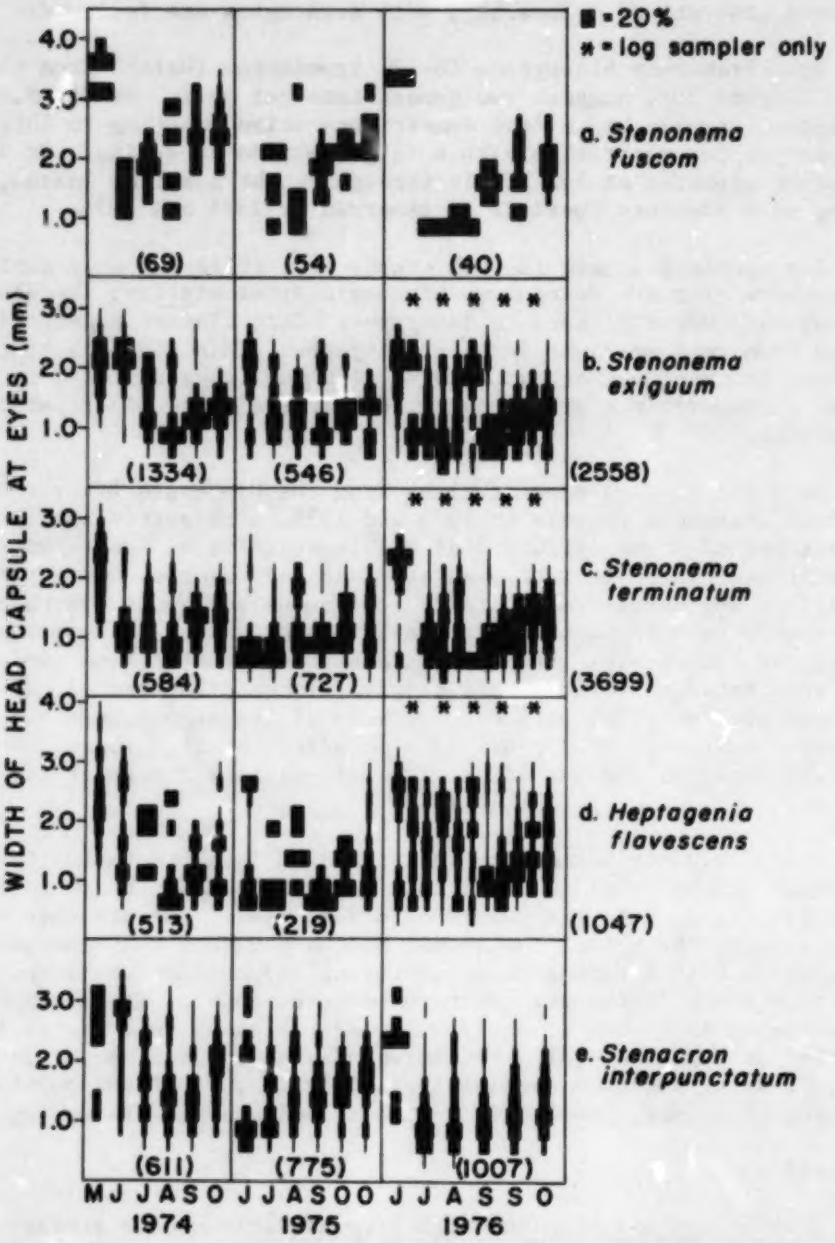

Figure 30. Percentage of nymphs collected at monthly (1974 and 1975) or twice-monthly (1976) intervals from the Wisconsin River and Rocky Run Creek. Numbers in parentheses indicate the total number of nymphs collected during the year.

previous generation). Possibly, both strategies are followed.

Size frequency histograms for *S. terminatum* (Walsh) from the Wisconsin River (Figure 30c) suggest two generations per year. As did *S. exiguum*, *S. terminatum* usually had a fast summer generation emerging in late summer, and a longer winter generation with a flight period in spring. In 1975, emergence appeared at low levels throughout the sampling season, rather than during more discrete periods as observed in 1974 and 1976.

Heptagenia diabasia (Burks) nymphs were collected only during June in low numbers from the downstream Wisconsin River station; therefore, their life history was difficult to interpret. Size classes present in June ranged from very small to very large nymphs. This suggests that emergence occurred in late June or early July, with some eggs hatching in spring. The number of generations present each year in the Wisconsin River could not be determined.

Data for *H. flavescens* (Walsh) from the Wisconsin River indicate spring and fall emergence periods in 1974 and 1975, with nearly continuous summer recruitment of young (Figure 30d). This suggests at least two successful generations. In 1976, increased abundance and samples taken twice each month from log substrates indicate continuous emergence and hatching. The discrepancy between samplers apparently is an effect of sampling interval rather than substrate. Data on log substrates, taken from only those dates when artificial substrates were sampled (1-month intervals), also suggest two separate emergence periods. Because of low summer populations in 1974 and 1975, multivoltinism could have occurred in all 3 years. The frequent sampling interval did not affect the life-history interpretations of other species.

Size-frequency histograms for *Stenacron interpunctatum* (Say) from Rocky Run Creek (Figure 30e) indicate that hatching usually began in late June, often continuing into the summer at a low level. The presence of a few, large animals throughout the summer months suggests that emergence was continuous, with a strong pulse in spring and another weaker pulse in late August or early September. Corresponding periods of growth appear, one beginning in late June or early July and extending into the fall, and possibly another preceeding the August-September emergence. In the study area, *S. interpunctatum* was multivoltine with most of the population hatching in summer, growing over winter, and emerging in spring.

Discussion--

Habitat and Distribution of Heptageniidae--Habitat preferences of the species found in this study were described by Flowers (1975). *Heptagenia diabasia, Stenonema integrum,* and *S. terminatum* are all typically found in medium to large, deep rivers, especially those with sandy substrates. *S. exiguum* is abundant in this habitat, but also thrives in smaller, rocky streams. *S. fuscom* and *Stenacron interpunctatum* are common in a wide range of lotic habitats; the latter is silt tolerant and is occasionally found on the rocky shores of lakes.

Lewis (1974) summarized the known distributions for *Stenonema* species. Most are widespread in the eastern and central forested regions of the United States, while *S. fuscom* is restricted to the Great Lakes area. *Stenacron interpunctatum* inhabits the entire eastern half of the United States and southern Canada. Distributions of some *Stenonema* species coincide, while others have little overlap. Distributions of *Heptagenia diabasia* and *H. flavescens* have not been studied extensively.

Temporal Variability (Annual)-- The life-history phenology of species changes from year to year. Some species (e.g., *Stenonema fuscom*) vary in time of hatching by only a few weeks, probably in response to prevailing environmental conditions such as temperature and photoperiod (Nebeker 1971). Other species are more variable in the timing of life-history events. For example, *S. terminatum* had a more extended, but low larval emergence in 1975 than in other years. Perhaps the extreme fluctuations in water level during 1975 influenced development and emergence of this species.

S. exiguum also varies considerably in the amount of time taken for development following oviposition. Young nymphs sometimes are caught well before the previous generation reaches full size and sometimes only after large nymphs have disappeared from the samplers. The possibility that part of an egg batch is delayed in hatching might explain how some nymphs could follow a life-history pattern typical of multiple cohorts, while others could exhibit true bivoltinism. Another possibility would be that delayed growth follows hatching. As noted, it could not be established definitely which of the life-history patterns, or both, occurs.

Few reports in the literature examine year-to-year variation in life-history patterns. McClure and Stewart (1976) suggested that changes could be made in the life cycle of the mayfly, *Chloroterpes mexicanus*, in response to changing environmental conditions, especially where brood overlap existed. Other authors documenting year-to-year variation in mayfly life cycles were Minshall (1967) and Brittain (1972). In these studies, a single generation per year was found and the time and duration of life-history events did not vary by more than 2 to 4 weeks each year.

Clifford (1970) suggested a mechanism for year-to-year variation when he found that *Leptophlebia* sp. in subarctic Canadian streams could emerge any time after reaching a certain size, though they often continued growing beyond that size. The effect was to accumulate nymphs capable of emerging until conditions for emergence were suitable.

Spatial variability (local and geographic)--Life-history data from this study were compared with those of other studies in Wisconsin (Table 29) and elsewhere in North America. Shaffer (1975) collected specimens at approximately monthly intervals from artificial substrate basket samplers in the Kickapoo River, a small stream in southwestern Wisconsin with a mud substrate and frequent riffles. Flowers (1975) collected Heptageniidae monthly, using a hand net, from various streams throughout the state. Some species collected in these studies differed only slightly in the timing and duration of life-history events. Others showed different numbers of

TABLE 29. COMPARISON OF LIFE HISTORIES OF HEPTAGENIIDAE FROM WISCONSIN

Species[+]	Schwarzmeier; Wisconsin River and/or Rocky Run Creek (1974-76)[§]	Flowers (1975): Wisconsin streams (1974)[§]	Shaffer (1975)[‡] Kickapoo River, Wisconsin (1974)[§]
Stenonema fuscum	1 univoltine winter brood	1 univoltine winter brood	Not present
S. integrum	1 univoltine winter brood	1 univoltine winter brood	Not present
S. exiguum	2 generations (multiple cohorts/bivoltine)	1 univoltine winter brood	Bivoltine
S. terminatum	2 generations (bivoltine)	1 univoltine	Bivoltine
Heptagenia diabasia	Spring emergence[¶] + hatching; number of generations unknown	2 generations (multiple cohorts)	Continuous summer[¶] emergence + hatching; number of generations unknown
H. flavescens	Bivoltine/multivoltine	2 generations	Not present
Stenacron interpunctatum	Multivoltine	Bivoltine	Multivoltine

[+] Species are arranged in order of increasing numbers of generations per year.
[†] Classifications designated by Schwarzmeier for data collected by Schaffer (1975).
[§] Year(s) of collection.
[¶] Incomplete data; or limited interpretation possible.

generations per year. For example, life histories of *Stenonema exiguum* and *S. integrum* from Rocky Run Creek and the Wisconsin River are consistent with results obtained by Flowers (1975). Data for *Stenonema exiguum, S. terminatum*, and *Stenacron interpunctatum* correspond to Shaffer's (1975) results, whereas Flowers (1975) found fewer generations per year for these species. A lack of complete data for *Heptagenia diabasia* and *H. flavescens* precludes comparisons.

Even though all these streams are exposed to similar climatic conditions, local differences could explain the variation in life histories. For example, streams sampled by Flowers usually had lower maximum (summer) temperatures than did the Wisconsin River, Rocky Run Creek, or the Kickapoo River. This suggests that, for some species, warmer temperature regimes allow faster development and more generations per year.

A comparison of Wisconsin data to heptageniid life histories reported from southern locations show that two species had more generations per year than did Wisconsin species. *Stenonema integrum* has two generations in the Ohio River basin (Lewis 1974), while *S. exiguum* is multivoltine in Florida (Pescador and Peters 1974) and other parts of the southern U.S. (Lewis 1974). *Stenacron interpunctatum* is multivoltine in all parts of its range where its life history has been studied (Peters and Warren 1966, Lewis 1974, Pescador and Peters 1974). *Heptagenia flavescens* had one spring emergence in Arkansas (Peters and Warren 1966), as compared to two or more generations in Wisconsin.

Other researchers have found that the time and duration of mayfly emergence varies from stream to stream. For many species, two general patterns exist. In warmer streams (in southern regions or at low altitudes) earlier emergence, and sometimes later hatching, may occur with no change in the number of generations per year (Macan 1957, Pleskot 1961, Maitland 1965, Minshall 1967), or the emergence period may be extended over a longer time period compared to emergence in colder streams (Lemkuhl 1968, Clifford 1969). These patterns suggest a possible mechanism for achieving more generations per year in warmer climates, as is found in many Heptageniidae. If emergence occurred early enough, additional time would be available for another generation to hatch and grow. If emergence of several generations extended over a long enough time period, overlap in generations could occur. Eventually, in the warmest climates, emergence and hatching could become nearly continuous (Corbet 1964).

The comparisons made from different streams and in different years in this study reveal patterns in the variability of life histories of Heptageniidae. Temporal variability occurs to some extent in most species. Some vary in the time of hatching and emergence by only a few weeks; others show greatly extended emergence in some years compared to others. Spatial variability is evident in species having more generations per year in warmer streams (either locally or in the southern part of their range). This seems to be further evidence that temperature is an influential factor regulating heptageniid life cycles. Possibly, these species also emerge earlier in warmer streams, although no adult collections were made to test this. Variability in life histories is an advantage to mayflies because it allows them to survive in a variety of conditions, whether within a single stream over time or over different local or regional climatic regimes.

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APPENDIX A

REVIEW OF LITERATURE ON ENTRAINMENT FROM COOLING LAKE INTAKE STRUCTURES

The topics of concern in Appendices A, B, and C are: (1) Entrainment from cooling lake intake structures; (2) acid rain; (3) alternative disposal of fly ash. Appendix A discusses the possible entrainment damage to fish and invertebrate populations at the Columbia site; possible damage appears minimal. In addition, a 1978 study of fish entrainment at the site by Swanson Environmental Inc. (1977) has revealed that fish loss due to the current water intake systems is minor. Acid rainfall, the topic of Appendix B, is not considered a potential problem for aquatic ecosystems at Columbia because of the high hydrogen ion buffering capacity that results from the calcareous nature of the drainage basin. The results of Appendix C indicate that the high pH of the ash expected from Unit II at Columbia will substantially reduce the pollution potential from a landfill. However, the landfill site must be chosen carefully to avoid direct connection with the ground water.

The effects of cooling water intake on aquatic systems have been studied at many power plants over the last 20 years. Although the studies differed in their approach, detail, and conclusions, four general areas of concern have emerged: (1) Removal of animals suspended or swimming in the water column; (2) mechanical injury via impingement upon intake screens or abrasion in pumps, pipes, and condensers; (3) the toxic effects of biocides used in reducing the fouling of pipe systems by microorganisms; (4) the various effects of thermal shock during condenser passage.

The removal of animals from the water column, including the impingement of adult and juvenile fish, has become the focus of a federally mandated monitoring program, pursuant to the requirements of Public Law 92-500. Freeman and Sharma (1977) conducted a survey of these programs, but a summary volume is not complete. The removal aspect of cooling water intake is relevant to the Columbia site; mechanical, toxic, and thermal aspects of entrainment do not apply. The Columbia station withdraws water from the artificial cooling lake to cool the superheated steam in the turbines. It is essentially a closed system, except that evaporative losses from the lake require a constant input from the Wisconsin River. The "make-up" water is presently pumped from the intake channel to the artificial lake by two 10,000-GPM pumps. Water is drawn down an intake channel that connects with the river approximately 3,000 ft from the cooling lake. The channel is protected by two bar-grilles and a fish conservation traveling screen.

Studies of mechanical injury and mortality during entrainment have been reported by Marcy (1973, 1976), Carpenter et al. (1974), Ginn et al. (1974), King (1974), Davies and Jensen (1975), and Polgar (1975). Several reviews such as those of Coutant (1970, 1971) and Hillegas (1977) have been published. Although survival of damaged organisms is often quite low, it does not appear that the number of organisms lost seriously effects the aquatic systems.

Biocides such as chlorine are usually used at such low concentrations that they pose no threat to entrained organisms or the receiving body of water (Marcy 1971, Bass and Heath 1975, Basch and Truchan 1976, Brungs 1976, Seegert and Brooks 1977). However, thermal shock, combined with small amounts of chlorine, has a greater effect than increased temperatures or chlorine levels alone (Ginn et al. 1974, Eiler and Delfino 1974).

Cooling system designers now use predictive tools to minimize impact. Curves and models can predict the amount of mechanical damage (Polgar 1975) and the extent of lethal and sublethal thermal effects (Coutant 1971) expected for a given intake design. Models have been developed by Goodyear (1977), Christensen et al. (1977), and others that forecast the effects of removal on given fish populations.

Potential Effects of Cooling Water Intake at the Columbia Site

The effects of entrainment of aquatic organisms from the Wisconsin River by the Columbia Generating Station differ from the effects seen at most other generating stations. At Columbia there is no direct return of the entrained water to the river. The analogy of the intake acting as a large predator on the river ecosystem (Coutant 1970) is more applicable than in "once-through" cooling situations. When assessing potential effects, researchers often draw a relationship between the percentage of water in the river used and the resulting effect on the river. However, organisms in riverine communities typically show "patchy" distributions (Whitton 1975) and larger organisms can either avoid the intake channel or electively swim into it.

Zooplankton and Drifting Macroinvertebrates--

Zooplankton are too small to be screened out of the intake pumps and are less able to avoid the influence of the pumping current than are larger animals. The percentage of total river flow removed as intake water at Columbia presently averages 0.3%, with a maximum of 1.08%. Assuming that the number of organisms entrained by the Columbia intake is proportional to the volume of river water used, no significant loss of invertebrates from the Wisconsin River is expected. Several other entrainment studies at U.S. power plants (King 1974, Davies and Jensen 1975, Hillegas 1977) did not demonstrate measurable effects in downstream plankton communities even where abundant data was available and generating stations diverted up to 30% of the river flow.

Adult and Juvenile Fish--

A 1-year study of fish entrainment at the Columbia site (Swanson Environmental Inc. 1978) reported the number, species, length, and reproductive condition of fish impinged on the temporary screen box unit and on the traveling screen unit currently in use. Sampling was conducted for a continuous 24-h period once a week. An estimated 14% of the total intake volume was sampled. The catch numbers were extrapolated to estimate total annual impingement as 668±387 fish per year (mean ± 90% confidence limits). The number of adult and juvenile fish impinged at Columbia are low and even if all impinged fish die, there should be no effect on the river system.

Fish Eggs and Larvae--

The Swanson Environmental, Inc. (1978) study also included sampling for fish eggs and larvae. Submersible pumps mounted behind the traveling screen unit pumped the sample water into 423-μ nets. Pump rates were sufficient to prevent fish from avoiding the sampler. Estimated annual entrainment of larval fish was 126,659±93,994 larvae per year (mean ±90% confidence limits). No northern pike or walleye larvae were caught in the samples. According to a summary of fish census data for the Columbia site (Wisconsin Department of Natural Resources 1973), northern pike and walleye spawn in the wetland adjacent to Duck Creek. The mouth of Duck Creek is located just upstream of the Columbia intake (Figure 1). Northern pike larvae and fry remain on the spawning marshes until they attain a size of 20 mm at 16 to 24 days after hatching (Franklin and Smith 1963). Although emigrating larvae of this size would not be able to avoid the intake current, the river currents may be strong enough in early spring to sweep larvae past the intake. Larval walleye are known to migrate from their spawning marshes in intermittent pulses over a 10- to 15-day period (Priegel 1970). By sampling once every 7 days, the period of walleye larval entrainment could have been missed. Walleye larvae also may avoid entrainment by staying in the main currents and bypassing the intake as they enter the Wisconsin River. Newly hatched walleye larvae emerging from similar spawning situations on the Wolf and Fox Rivers in Wisconsin tended to stay in the strongest currents until they reached more lacustrine situations where zooplankton were abundant (Priegel 1970).

In summary, as long as the Columbia intake continues to remove a small percentage of the river flow, no measureable effects of entrainment on the river system are expected. An exception might occur when organism distribution is patchy near the intake and a significant portion of one year-class (i.e., walleye larvae) is entrained. Aside from acting as a predator by removing organisms from the Wisconsin River, the usual types of entrainment effects (mechanical, toxic, and thermal) do not apply to the Columbia station.

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APPENDIX B

REVIEW OF LITERATURE ON ACID RAIN

Recent studies in North America and Europe have documented the occurrence of rains with a pH ranging from 2.1 to 5.0 (Likens and Bormann 1974, Beamish 1974, Dickson 1975, Beamish 1976, Schofield 1976). Rainwater is normally slightly acidic (pH 5.7), a result of the equilibrium reaction between atmospheric carbon dioxide and water that forms carbonic acid (H_2CO_2). However, both natural and anthropogenic processes can add three strong mineral acids--sulfuric (H_2SO_4), nitric (HNO_3), and hydrochloric (HCl)--to atmospheric water with a resulting sharp decrease in pH (Gorham 1976). The most predominant of these acids is H_2SO_4 which can be formed in substantial amounts from the sulfur dioxide (SO_2) produced as sulfur in fossil fuels oxidizes during combustion. Coal normally has between 1 and 3% sulfur, but the percentage can go as high as 6%. Of less importance are HNO_3 and HCl, which also are produced by fossil fuel combustion through the oxidation of organic nitrogen and chlorine, respectively. These acids may then enter aquatic systems through rainfall or, in northern latitudes, through ice and snow runoff.

The work of Cogbill and Likens (1974) illustrates that acid rain is likely to remain a problem in certain areas. By graphing isolines of rainfall pH falling over the eastern U.S., they have shown a dramatic increase in the geographic area affected by acid rain, as well as an increase in rainfall acidity for 1956-66.

The initial effects of acid input into lakes and streams depends largely on edaphic characteristics that determine buffering capacity. All waters affected by acid rain are in areas that are geologically highly resistant to chemical weathering and usually have a low concentration of major ions--particularly biocarbonate (HCO_3)--resulting in a specific conductance less than 50 mhos/cm (Wright and Gjessing 1976). Acid rainfall into weakly buffered systems causes the bicarbonate ion to be lost and then replaced by sulfate. Hence, sulfate is the major anion in acid soft water, whereas bicarbonate predominates in non-acid soft water. Acid lakes frequently contain elevated aluminum and manganese concentrations that are attributed to dissolution from surrounding soils. Elevated levels of other heavy metals (Pb, Zn, Cu, and Ni) may also exist downwind of major base metal smelters (Van Loon and Beamish 1977).

Ecological studies concerned with acidification of aquatic ecosystems have focused on fish population, since the loss of an exploitable fish population is the most noticeable and economically important consequence of acid rain. Fish loss is reported to be a gradual process, resulting not from acutely lethal pH changes, but from the failure to recruit new year classes into the population (Beamish 1974). At pH values above the lower lethal level, laboratory and field studies have demonstrated interference with spawning (Mount 1973, Beamish 1976). The presumed mechanism responsible for reproductive failure is disruption of normal calcium metabolism that prevents females from releasing their ova (Beamish 1976). Long-term effects of acidification on fish populations were summarized by Beamish as follows: (1) Failure to spawn; (2) low serum Ca^{++} levels in mature females; (3) appearance of spinal deformities; (4) decreases in the average size of year-classes; (5) reduction in population size, (6) disappearance of species from lakes.

Studies have indicated a genetic basis for acid tolerance at the species level (Gjedrem 1976, Robinson et al. 1976, Schofield 1976). Selective breeding of acid-tolerant fish strains has been proposed as a means of stocking waters that have lost their natural populations. However the observed rates of population extinction indicate that acidification has been too rapid for natural selection processes to effectively maintain fish populations under natural conditions.

The effects of acid rain on aquatic organisms such as microdecomposers, primary producers, zooplankton, and zoobenthos are less conspicuous, but are equally as serious as damage to fish. Studies in six Swedish lakes, where the pH decreased by 1.4 to 1.7 pH units in the last 40 years, have demonstrated an inhibition of bacterial decomposition with a resultant abnormal accumulation of coarse organic detritus (Hendrey et al. 1976). Rooted macrophytes, zooplankton, and benthic invertebrates also are stressed by acidification of waters (Hendrey et al. 1976). Table B-1 summarizes some of the effects of pH on aquatic organisms.

TABLE B-1. SUMMARY OF pH EFFECTS ON AQUATIC ORGANISMS

pH	Effect	Reference
< 3.5	Unlikely that fish can survive for more than a few hours; A few invertebrates (midges, mosquito, caddisfly) have been found; Few plants (only mosses and algae) have been found.	European Inland Fisheries Advisory Committee (1969); Lackey (1938) Hendrey et al. 1976a
3.5–4.0	Lethal to salmonids and bluegills, limit of tolerance of pumpkinseed, perch, and pike, but reproduction is inhibited; Cattail (*Typha*) is the only higher plant.	U.S. Environmental Protection Agency 1973
4.0–4.5	Only a few fish species survive, including perch and pike; Lethal to fathead minnows; flora are restricted; Some caddisflies and dragonflies are found and midges are dominant.	U.S. Environmental Protection Agency 1973
4.5–5.0	Salmonids may survive, but do not reproduce; Benthic fauna are restricted; mayflies are reduced; Fish populations are severely stressed;	U.S. Environmental Protection Agency 1973
	A viable fishery is non-existent; Snails are rare or absent;	Hendrey et al. 1976a
	The fish community is decimated with virtually no reproduction;	Beamish 1974, 1975
	White suckers and brown bullheads fail to spawn, but perch do spawn.	Beamish 1975

Continued

Table B-1. Continued

pH	Effect	Reference
5.0-6.0	Rarely lethal to fish except some salmonids, but reproduction is reduced; Larvae and fry of sensitive species may be killed; Bacterial species diversity is decreased, benthic invertebrates are reasonably diverse, but sensitive taxa such as mayflies are absent and molluscs are rare;	U.S. Environmental Protection Agency 1973
	Fathead minnow egg production and ability to hatch are reduced;	Mount 1973
	Smallmouth bass, walleye and burbot stop reproducing;	Beamish 1976
	Roe of roach (*Rutelus rutelus*) fail to hatch.	Milbrink et al. 1975
6.0-6.5	Unlikely to be harmful to fish unless free CO_2 exceeds 100 ppm; Good invertebrate fauna except for reproduction of Gammarus and Daphnia; Aquatic plants and microorganisms relatively normal.	U.S. Environmental Protection Agency 1973
6.5-9.0	Harmless to fish and most invertebrates although 7.0 is near the lower limit for Gammarus reproduction; Microorganisms and plants are normal; however, toxicity of other substances may be affected by pH shifts within this range.	U.S. Environmental Protection Agency 1973

Although pH measurements of rainfall in the vicinity of the Columbia Generating Station have not been made, it appears unlikely that acid rainfall will noticeably affect nearby aquatic ecosystems for the following reasons: (1) The Wisconsin River, Rocky Run Creek, and nearby waters are well-buffered systems with total alkalinities in the range of 80 to 133 mg/liter $CaCO_3$ and conductivities of 178 to 273 mhos/cm; (2) winds are predominately from the west and south (Stearns et al. 1977), therefore power plant emissions should miss most of the nearby aquatic systems that are mainly west and south of the plant; (3) the current pH of the Wisconsin River (7.6 to 8.2) and Rocky Run Creek (7.6 to 8.2) are well within the recommended safe range of 6.5 to 9.0 for natural waters and have not changed noticeably since the plant began operating in 1975.

The effect of additional sulfur emissions when Columbia II begins operation should be considered. Also to be accounted for are the contributions, if any, of the Columbia plant emissions to acid rainfall over distant waters, such as northern Wisconsin lakes, some of which are poorly buffered and more subject to acidification.

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APPENDIX C

REVIEW OF LITERATURE ON ALTERNATIVE DISPOSAL OF FLY ASH

The increased national emphasis on the use of coal to meet energy requirements may double 1975 coal ash production levels by the year 1995 (PEDCo-Environmental, Inc. 1976). Annual coal ash production is currently estimated to be 61.9 x 10^6 tons (Davis and Faber 1977) and may be 100 x 10^6 tons by 1985 (Harriger 1977). About 20% of the ash is used for commercial purposes such as cement, asphalt and concrete, fertilizer, fire control, road bed stabilizer, soil aeration, and sanitary landfill cover PEDCo-Environmental 1976, Theis 1976a, Harriger 1977). There is continuing research into additional uses for coal ash, such as water reclamation, sewage sludge conditioning, and supplementation of soil sewage micronutrients (Theis 1976a, Furr et al. 1977). Fly ash and lime cause the precipitation of phosphorus from natural waters and the ash seals the nutrient in the sediment; however, the side effects of such treatment may be severe (Theis and DePinto 1976). Fly ash concentrations of 10 to 20 g/liter were toxic to Stone Lake (Michigan) fish; high pH, dissolved oxygen depletion, heavy metal release, and physical clogging and crushing of organisms are other effects that have not been adequately investigated. Fly ash applied to soils can neutralize acid soils and supply calcium and trace elements (PEDCo-Environmental 1976); however, the high conductivities of fly ash-water solutions may increase salt concentrations to injurious levels for many sensitive crops (Olsen and Warren 1976). Theis (1976a) suggests the extraction of quantities of rare metals from ash for industrial re-use; for example, a generating station producing 260 tons of ash/day could provide approximately 53.2 kg As/day, 5.2 kg Pb/day, 5.0 kg Cu/day, 49 kg Zn/day, 12.3 kg Cr/day, 730 g Cd/day and 18.9 g Hg/day.

Presently, more fly ash is produced than is demanded by commercial users (Theis 1976a). The average rate of ash production is 0.5 kg/kWh (PedCo-Environmental, Inc. 1976) and increased coal use will increase the amount of ash to be disposed of. The New Source Performance Standards (NSPS) applicable to new power plants prohibit discharges from ash settling ponds to enter natural waters (Dvorak and Pentecost 1977). To comply with these regulations, ash from Unit II of the Columbia Generating Station is being held in a segregated portion of the ash basin until a site for permanent land disposal is found.

Many concerns remain regarding the landfill disposal of coal ash. In addition to the continued threat of surface contamination due to precipitation and overland runoff, ground-water contamination and landfill erosion are significant concerns. Although many of the principles of sanitary landfilling are applicable if the different nature of the contaminants is considered, an expanded study of coal ash landfills is needed. Information on the leaching and mobility of ash trace constituents is limited (Dvorak and Pentecost 1977) and because the disposal method is recent, there is little knowledge of the long-term effects of such disposal. Studies are needed for the creation of standards for land disposal of toxic substances, which is virtually unregulated at the federal level (Fields and Lindsey 1975).

The potential for ground-water contamination by leachate produced when water percolates through a coal ash landfill draws the most widespread concern. High salt concentrations in leachate may be a significant problem, especially if the leachate reaches ground-water supplies that are already high in salt. Increased pH due to ash leachate may be a localized problem (Olson and Warren 1976); however, pH is important because it affects metal solubilities and adsorption. This potential for metal and other trace element contamination has received the greatest attention and concern.

The ability of the soil to attenuate contaminants in the leachate is of primary importance in preventing ground-water contamination by any kind of landfill. Waldrip (1975) found that inorganic and organic materials from sanitary landfill leachate are adsorbed by the soil and that many desirable ions replace undesirable ions in an ion exchange process. He concluded that most ground-water contamination is limited to the immediate vicinity of the landfill because of the slow movement of the groundwater. The low velocity allows sufficient time for ion exchange, dilution, and dispersion to occur. The landfill contribution to ground-water supply is significantly diminished within a few hundred feet of the landfill.

Griffin et al. (1976) studied the attenuation of metals and other leachate constituents run through laboratory sediment columns. Clay was relatively poor in reducing concentrations of Cl^-, Na^+, and water soluble organic compounds, but K, NH_4, Mg, Si, and Fe were moderately reduced in concentration, probably by cation exchange with Ca in the soil. Low leachate concentrations were strongly attenuated by small amounts of clay, possibly because of precipitation of the metals upon formation of metal hydroxides or carbonates (caused by high pH and high bicarbonate concentration in the leachate). Low leachate concentrations of Al, Cu, Ni, Cr, As, SO_4, and PO_4 precluded interpretation for those substances. Suarez (1974) describes the chemical reactions involving metals leached from sanitary landfills and discusses their relationship with Eh, pH, and dissolved oxygen.

A comparison of fly ash landfill investigations is necessary to determine the applicability of sanitary landfill results to a landfill designed for fly ash. Theis (1976b) and Theis and Marley (1976) discuss the potential for ground-water contamination from land disposal of fly ash. They determined that the important characteristics of ash are initial trace metal concentration, acid-base characteristics, fly ash concentration in the aquatic system, and the size fraction distribution of the ash. A combination of field and laboratory studies demonstrated that Cr, Cu, Hg, Pb, and Zn are released from leachate in insignificant amounts or are rapidly sorbed onto soil particles; however, As, Ni, and Se occurred in ground water at higher concentrations and appeared able to migrate a greater distance. Sorptive processes could explain the metal leachate behavior in the initial desorption of metals from the ash into water and subsequent adsorption onto the soil phase.

The investigation of a landfill for fly ash from the combustion of eastern U.S. coal (Harriger 1977, Harriger et al. 1977) is the most comprehensive study to date. The presence of clay-rich soil was determined to be the most important factor affecting water quality. Other factors include composition and quality of the ash, duration of exposure to leaching, pH, oxidation conditions, and surface and ground-water flow patterns. Clay soils were relatively impermeable and were found to adsorb or exchange large quantities of ions. Ground-water wells away from the landfill were lower in concentrations of many trace substances, attesting to the benefits of leachate percolation through the soil. Landfill wells often had concentrations of As, Se, Fe, Mn, and SO_4 above the U.S. Public Health Service drinking water recommendations. Landfill wells also exhibited higher concentrations of Zn, Ca, Cr, Cu, Mg, and K than the off-site wells; however, Ca, Cr, and Cu were fairly low because of low concentrations in the ash itself, good attenuation by clay, and the prevailing pH conditions.

Analysis of surface waters (streams flowing across the landfill, runoff from the landfill, and ponds formed from precipitation) indicated few effects of the landfill once the water left the site. A stream enclosed by pipe as it crossed the site appeared to receive some ground-water and ash leachate seepage downstream. Concentrations of Fe, Mn, and SO_4 exceeded drinking water standards, but decreased rapidly downstream. Calcium, Cd, Cu, Fe, Mg, Na, Se, Zn, and SO_4 levels were higher and pH was lower in ponds on the landfill (especially those with exposed ash deltas) than in control ponds away from the site. Metal concentrations were higher in the sediments of the landfill ponds, indicating that the contaminants were precipitating out of the water. Metal concentrations were high in runoff water from the landfill and low concentrations of Cr, Cu, and Zn in the ground water evidenced attenuation by clay and restricted metal mobility in ground water. This indicates the need to contain surface runoff to permit these mechanisms to operate.

The pH and oxidation states of materials in the landfill influence the effectiveness of the attenuation mechanisms. The solubility of most metal ions increases at lower pHs (Harriger 1977), and thus in acidic leachate metals are not removed as readily by the attenuation processes. Generally, high pH greatly decreases solubility and only Zn and Cd are considered soluble in the pH range 7 to 8.5 (Theis 1976a). Most Cr is released from ash into the leachate at pH 3, although some is released at pHs of 6, 9, and 12 (Theis and Wirth 1977). Iron and Mn precipitate at pH > 7.5 (Harriger 1977). Fields and Lindsey (1975) conclude that low pH affects ion exchange and that adsorption properties of soil-clays are more effective in adsorbing most metals when the pH is high, although a low pH is best for adsorption of organics. They state that it is best to maintain landfill soils at pH 7.0

to 8.0. Frost and Griffin (1977) found, however, that As and Se adsorption by clays is decreased at high pH. Oxidation causes the formation of iron oxides and hydroxides; these precipitate from the leachate and can adsorb other ions (Harriger 1977), thus increasing the purification capacity of the soil.

The relative amounts of lime and amorphous iron oxides in the ash determine the pH of the leachate. Western coals have high amounts of lime (Theis and Wirth 1977), which account for the basic nature of the ash from the Columbia statiow. The greatest environmental concern with low pH ashes is the large amount of surface leachable Fe (Theis and Wirth 1977). Theis (1976a) states that a greater amount of metal probably will be released from ash into ground water than into surface water. This is because of the lower pH and high CO_2 content of ground water and the consequently greater likelihood of ion exchange from ash into this water.

Research continues into the principles of site selection and design to reduce the threat of ground and surface water contamination as much as possible. Little is known about the potential environmental effects of landfills in Wisconsin (Zaporozec 1974) and there have been few long-term studies of solid waste disposal in the United States. Leachate production occurs even in well-designed landfills, especially in humid areas such as Wisconsin (Zaporozec 1974, Fields and Lindsey 1975); however, this production can be minimized or controlled with proper site selection and design.

Many investigators suggest the use of liners, either impervious to retain all leachate, or permeable ones to supplement the ability of the soil to attenuate pollutants (Fields and Lindsey 1975, Griffin et al. 1976, PEDCo-Environmental, Inc. 1976, Dvorak and Pentecost 1977). Where clay in native soils is insufficient, a clay liner can satisfactorily mitigate the contamination threat. It has been suggested that ash landfills may have the capacity to seal themselves against leachate loss. As soluble CaO moves into the soil and forms $CaCO_3$, the permeability of the soil may be significantly reduced (Olsen and Warren 1976). Fly ash is often deliberately applied to sanitary landfills because of its moisture absorbing characteristics (PEDCo-Environmental, Inc. 1976).

Another suggestion to reduce the potential of contamination is vegetating the landfill to reduce erosion by wind or water. Harriger (1977) found that erosion remained a problem when the ash was covered with bare soil. PEDCo-Environmental, Inc. (1976) suggests the use of species tolerant to high pH, boron, and salt. Recommendations for sanitary landfills in southern Indiana include: Use of upland sites to avoid runoff from upland areas; use of sites whose soils or intervening materials have high exchange and adsorption capacities; use of leachate lagoons to prevent surface-water contamination, use of sites where the water table is much below the bottom of the waste; avoiding areas subject to flooding (Waldrip and Ruhe 1974). PEDCo-Environmental, Inc. (1976) presents a detailed discussion of geological, chemical, and engineering aspects of landfill site selection and design. A literature review by Heidman and Brunner (1976) lists references concerning site location, investigation, monitoring, and management for sanitary landfills. Much of the information in both reports can be applied to coal ash landfills.

Several states and agencies have criteria and regulations that should be considered in the construction of coal ash landfills in Wisconsin. The California State Water Resources Control Board (1975) lists the following: (1) Underlying geological formations with questionable permeability must be permanently sealed or ground-water conditions must prevent hydrologic continuity; (2) leachate and subsurface flow must be self-contained; (3) sites must not be located over zones of active faulting; (4) limitations are applied if the area is in a 100-year (or more frequent) flood-frequency class. A study for the U.S. Environmental Protection Agency Battelle Memorial Institute (1973) recommends the following criteria: (1) Low population density; (2) low alternate land use value; (3) low ground-water contamination potential; (4) away from flood plains, excessive slopes, and natural depressions; (5) soil with high clay content; (6) adequate distance from human and livestock water supplies; (7) areas of low rainfall and high evaporation rates, where possible; (8) sufficient elevation over the water table; (9) no hydrologic connection with ground or surface water; (10) use of encapsulation, liners, waste detoxification, or solidification/fixation, where necessary; (11) adequate monitoring. Consideration of all these suggestions will significantly reduce, if not avoid entirely, the adverse effects that a fly ash landfill might have on environmental quality.

It appears that the high pH expected from Columbia II will substantially reduce the pollution potential from a landfill. However, the landfill site must be chosen carefully to avoid direct connection with the ground water. A clay or other type of liner will probably be beneficial, if not required, to avoid ground-water contamination. Pipes to collect and recirculate leachate should be used if there is any likelihood of less than complete metal attenuation by the time the leachate reaches the ground water.

SUMMARY

1. Fly ash may be used commercially for a variety of purposes, but supply probably will continue to exceed demand (PEDCo-Environmental 1976, Theis 1976a, Theis and De Pinto 1976, Harriger 1977).

2. Although recent air and water pollution standards prohibit the discharge of ash or its leachate into surface waters, considerable concern has arisen over the potential adverse effects of the dry disposal of fly ash in landfills.

3. Metal and trace element contamination of water, particularly ground water, is the most serious concern. Soils vary widely in their abilities to attenuate these pollutants.

4. Clay soils have the greatest capacity for metal adsorption and ion exchange (Griffin et al. 1976, Harriger 1976, Theis 1976b, Theis and Marley 1976).

5. Because of these mechanisms, and due to dilution and dispersion in slow-moving ground water, most ground-water contamination is limited to the immediate vicinity of the landfill (Waldrip 1975, Harriger 1977).

6. With proper precautions, direct surface-water contamination is usually minimal (Harriger 1977). Appropriate precautions include containment of surface runoff and avoidance of low sites and steep slopes.

7. Better attenuation of metals is usually obtained when the leachate has a high pH. Metal solubilities are reduced and clay properties are improved under these conditions (Fields and Lindsey 1975, Theis 1976a, Harriger 1977). Fortunately, the western U.S. coal burned at the Columbia Generating Station produces basic conditions in its ash.

8. Where natural soils are not sufficient, clay or impervious liners should be applied to the landfill (PEDCo-Environmental 1976, Dvorak and Pentecost 1977). Fly ash appears to have some capacity to form a seal itself (Olsen and Warren 1976).

9. Other recommendations to reduce the potential environmental contamination include covering with soil, encouraging vegetation, containing leachate, adequate monitoring, and avoiding sites with high ground water, flooding potential, active faulting, or low elevations.

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APPENDIX D

LITERATURE REVIEW: THE DYNAMICS AND EFFECTS OF CHROMIUM AND OTHER METALS IN ORGANISMS

Many metals are essential components of living organisms in trace amounts. Metals such as ZN, Fe, and Cu are necessary constituents of many enzymes and pigments (Prosser 1973). There is recent evidence that annimals require chromium in their diets for normal glucose metabolism and that dietary Se may offer protection against chemical carcinogens, methylated mercury, and Ca (Allaway 1975). It is when concentrations exceed the necessary or beneficial levels that organisms may be adversely affected by metals; this may be occurring in the stream system receiving coal ash effluent with its elevated metal concentrations.

Recently, there has been increased concern over the effects of elevated environmental metal levels on individual organisms and, particularly, over the ramifications of increased metals in food chain relationships and bioaccumulation by higher trophic levels. Bioaccumulation is well known in some metals, such as Hg and Cd. Both metal-susceptible and metal-tolerant organisms may be hazards to their consumers in higher trophic levels. Kania and O'Hara (1974) found that mosquitofish (*Gambusia*) exposed to sublethal concentrations of Hg were less able to escape predation by bass. The mosquitofish with the highest metal concentrations would be the most heavily consumed, leading to increased metals in the food chain. Highly resistant organisms could accumulate very high metal concentrations before being preyed upon or dying and being consumed by detritivores; crayfish may be such a hazard. Gillespie et al. (1977) determined that *Orconectes propinquus* is highly resistant to Cd and could contribute significant amounts to the next trophic level. Crayfish, fed on by many species of fish, amphibians, reptiles, birds, and mammals are important in food webs (Neill 1951). Davis and Foster (1958) report that food chains tend to select for essential elements. This is of limited usefulness, however, since the majority of common metals are essential in small amounts and toxic in larger concentrations. The threat of bioaccumulation may not be as great for chromium as for other metals, however, since Sather (1966) found that the lower trophic levels (algae, sponges, and snails) concentrate more chromium than fish and crayfish.

Chromium toxicity and dynamics are highly dependent on chemical form and oxidation state. These parameters govern the behavior of chromium in the Columbia ash drainage system. Chromium has four oxidation states: Cr^{+2} and Cr^{+5} are rare in nature; Cr^{+3} and Cr^{+6} are most common. Hexavalent chromium salts are very water soluble (up to several g/liter) while most trivalent salts (including the very common hydrous oxides) are insoluble and thus exist in very low concentrations in the pH of natural waters (Foster 1963, Schroeder 1973). Heat, organic matter, and chemical reducing agents can reduce Cr^{+6} to Cr^{+3} (Foster 1963). The hexavalent form is widely recognized to be highly toxic, with the trivalent form moderately toxic to not toxic (Foster 1963, Mathis and Cummings 1973, Allaway 1975). Allaway (1975) found no reports of toxicity for dietary Cr^{+3}. Chromium entering natural waters is usually in hexavalent form, but is rapidly reduced to Cr^{+3} precipitated, and sorbed by the sediments. This reduction is usually due to organic matter or Fe^{+2} (with the Cr^{+3} then sorbed by the $Fe(OH)_3$ precipitate) (Schroeder 1973). These reactions are occurring in the ash pit drain because the coal ash effluent has a high Fe content. Thus, most chromium moves to the sediment in the trivalent form; whatever remains dissolved in the water is most likely Cr^{+6}. Hexavalent chromium is probably reduced to Cr^{+3} in living organisms, but there is no way to test this hypothesis since chromium cannot be extracted without affecting its oxidation state (Schroeder 1973). Huffman and Allaway (1973) indicate that Cr^{+6} is changed to Cr^{+3} in the stomachs of rats, and it is not easily absorbed by the intestine at neutral pHs.

There is disagreement over the relative importance of food and water in the uptake of metals by aquatic organisms. However, the mechanisms of uptake, transport, elimination, and regulation are becoming lucid. Davis and Foster (1958) report that although absorption and adsorption from water are important in the bioaccumulation of radioisotopes, the food chain is the most important factor. For animals that accumulate substances by ingestion, the concentration in the body will fluctuate with metabolic rate. Pentreath (1973) determined that the direct accumulation of zinc-65 and manganese-54 from water was small in comparison with dietary uptake by the plaice (*Pleuronectes platessa*). Freshwater animals appear relatively impermeable to Zn, thus all Zn is normally obtained from food and eliminated in the feces with the hepatopancreas regulating uptake, elimination, and transport (Bryan 1966, 1967). Bryan also found that this results in high Zn concentrations in crayfish hepatopancreas and stomach fluids. Concentrations remained constant in muscle, even with high amounts in the water, indicating that the absorbed Zn is probably returned to the blood. Food is also more important than water as a route for zinc uptake in marine crabs (Bryan 1966).

Bryan and Hummerstone (1971, 1973) found that Nereis accumulates Cu, zinc, and cadmium from water and ingested sediment; water is the primary route for Zn and Cd. Copper accumulation is probably unregulated since body concentrations are related to the concentration in the sediment. Tissue Zn concentration remains constant despite environmental concentration, indicating some degree of regulation. Odum (1961) reported that arthropods acquire Zn from water or food and that there are two pools in the body: 1) Unassimilated and rapidly lost with concentration dependent on the aqueous environment; 2) assimilated and excreted slowly at a rate proportional to metabolic rate. Fowler et al. (1970) tested Zn uptake in euphausiids and determined that accumulation occurred in similar tissues regardless of mode of uptake, except that none appeared in the exoskeleton after ingestion. The areas of localization were therefore independent of mode of uptake, but the quantities were different.

The crustacean exoskeleton is relatively impervious to many ions in solution (Wiser and Nelson 1964). Cobalt accumulation was greater in small crayfish than in larger crayfish per gram of body weight because they have a relatively greater proportion of surface area on which adsorption can occur. The integument had the highest cobalt concentrations, followed by the hepatopancreas. Metal elimination occurred at a slower rate than uptake. Metal-tolerant isopods (*Asellus meridianus*) accumulated Cu and Pb from food and water (Brown 1977), but there was no evidence that non-tolerant *Asellus* accumulated the metals from food. The non-tolerant animals also did not survive the exposure and had much smaller proportions of Cu in the hepatopancreas than did Cu-tolerant animals. On this basis, Brown proposes two possible tolerance mechanisms: Improved metal storage; improved metal detoxification.

Little work has been done to determine the importance of ingestion as a mechanism of chromium uptake. Uptake of Cr^{+6} did not occur even when it was placed directly in the stomachs of rainbow trout (*Salmo gairdneri*) (Knoll and Fromm 1960). The gills were the primary site of uptake from water due to differences in concentrations across the membranes. Blood maintained a concentration similar to that of the water, while all tissues except muscle exceeded environmental concentrations. In uncontaminated water, chromium elimination was rapid from all tissues except spleen.

Rats absorbed Cr^{+3} poorly in the intestine because of its low solubility at neutral pHs (Huffman and Allaway 1973). Fasted rats absorbed 6% of the Cr^{+6} they ingested. Acid conditions in their stomachs caused Cr^{+6} to be changed to Cr^{+3}. Tissue uptake was greatest in the liver, kidney, and blood (MacKenzie et al. 1959).

A marine polychaete (*Hermione hystrix*) placed in sea water containing $Cr^{+3}Cl_3$ exhibited tissue accumulation only on the body surface and in the digestive tract. When exposed to $Cr^{+6}O_4$, however, there was a small amount of passive tissue accumulation, which depended on water concentration (Chipman 1966). Which uptake route was most important was not evidenced.

Chromium uptake from water apparently occurs through the gills and is transported by the blood. This is reported for lobsters (Van Olst et al. 1976), which apparently regulate uptake of essential and non-essential metals, for crabs (Sather 1966), where the gills regulate chromium absorption dependent on oxidative phosphorylation and carbonic anhydrase action, and for largemouth bass, *Micropterus salmoides* (Fromm and Schiffman 1958). Elimination occurs via the gills in lobsters (Van Olst et al. 1976) and partially via the liver in fish (Fromm and Schiffman 1958).

The effects of many heavy metals on organisms are well documented (Becker and Thatcher 1973, Eisler 1973, Eisler and Wapner 1975.) Most laboratory documentation has been concerned with lethal effects and acute toxic limits (Table D-1 summarizes these for chromium), but there are some studies of sublethal effects (Table D-2 for chromium). Sublethal effects are varied and depend on the specific metals and organisms involved. Copper retards growth and development and damages tissue in crayfish at levels as low as 0.06 mg/liter (Hubschman 1967a, 1967b). Mercury affects the metabolic and swimming rates of larval crabs (DeCoursey and Vernverg 1972). Chromium irritates and causes pathological changes in the digestive tract a well as reducing oxygen consumption and possibly acting as a protein coagulant (Fromm and Schiffman 1958, Cheremisinoff and Habib 1972). An *in vitro* study (Buhler et al. 1977) indicated that trout enzymes are fairly insensitive to Cr^{+6} inhibition, but they, as well as Kuhnert et al. (1976), found significant enzyme reductions in several rainbow trout (*Salmo gaerdneri*) tissues upon *in vivo* exposure. Chromium appears to be different from most metals because it does not bind to the gill epithelia and mechanically interfere with respiration (Fromm and Schiffman 1958, Buhler et al. 1977).

Many factors affect the degree of toxicity and some of these factors pertain to the organism itself. Raymont and Shields (1963) suggest that resistance is probably attributed to permeability of the gut and body wall, composition of body tissue, rates of excretion, and size. Adaptation to Zn by *Nereis* is probably a result of reduced body surface permeability and an increased ability to excrete Zn, while Cu tolerance appears to result from a complexing system which detoxifies and stores Cu in the epidermis and nephridia (Bryan and Hummerstone 1971, 1973). Juvenile organisms are usually more susceptible than mature individuals (Hubschman 1967b, Doyle et al. 1976, Van Olst et al. 1976). The relative tolerance of fish and invertebrates to metals is controversial. Mathis and Cummings (1973) state that fish are less affected by metals, while Warnick and Bell (1969) conclude that fish are more susceptible. Many environmental factors affect toxicity--water hardness, temperature, and osmotic concentration of the medium (Bryan and Hummerstone 1971, 1973, Zitko and Carson 1976). Bryan (1971) presents the following table to illustrate the variety of factors affecting the toxicity of metals to aquatic organisms:

- Form of metal in water
  - Soluble
    - Ion
    - Complex
    - Chelate
    - Compound
  - Particulate
    - Precipitate
    - Adsorbed
- Presence of other metals or poisons
  - Antagonistic effects
  - Additive effects
  - Synergistic effects
- Factors influencing physiology of organism and perhaps form of metal in water
  - Salinity
  - Temperature
  - Dissolved oxygen
  - pH
  - Light?
- Condition of the organism
  - Stage of life history
  - Changes in life cycle (e.g., molt)
  - Size
  - Activity
  - Acclimation to metals

All of these factors may be operating in the ash effluent disposal system of the Columbia Generating Station, affecting not only toxicities but sublethal responses of organisms as well.

TABLE D-1. SUMMARY OF WORK DONE TO DETERMINE CONCENTRATIONS OF CHROMIUM THAT ARE LETHAL TO ORGANISMS

Form	Concentration	Effect	Organism	Other	Reference
Cr^{+3}: $CrCl_3$ $6H_2O$	2.0 mg/liter	3-week LC-50	*Daphnia magna*	Hardness: 45 mg/liter	Biesinger and Christensen (1972)
Cr^{+6}: Na_2CrO_4	0.21 mg/liter	100 h TL_m	*Daphnia magna*	Adding several Na compounds prolonged survival	Dowden and Bennett (1965)
Cr^{+6}: $K_2Cr_2O_7$	0.7 mg/liter	2-day toxic threshold	*Daphnia magna*		Bringmann and Kuhn (1959)
Cr^{+3} K-chromic	42 mg/liter	2-day toxic threshold			
Cr^{+6}	1.0 mg/liter	3-week toxic	*Nereis*	Marine	Raymont and Shields (1963)
	0.6-0.7 mg/liter	toxic threshold for longer tests			
Cr^{+6}: $K_2Cr_2O_7$	280 mg/liter	48-h TL_m	*Hydropsyche* larvae	Soft water	Roback (1965)
	3.5 mg/liter		*Stenonema rubrum* larvae		
Cr^{+6}	Various	Various	*Homarus americanus*	Larvae more sensitive than juveniles and adults	Van Olst et al. (1976)
Cr^{+3}	12.1, 9.3	24 h TL_m, 96 h TL_m (mg/liter)	*Nais*		Rehwoldt et al. (1973)
	6.4, 3.2		*Gammarus*		
	58, 50		caddisfly		
	46, 43.1		damselfly		
	16.5, 11.0		*Chironomus* sp.		
	15.2, 12.4		*Amnicola* sp. eggs		
	10.2, 8.4		*Amnicola* sp. adults		

Continued

TABLE D-1. Continued

Form	Concentration	Effect	Organism	Other	Reference
Cr^{+6}	5.0 mg/liter	40% kill-15 days	*Salmo gairdneri*		Fromm and Stokes (1962)
	10-12.5 mg/liter	80% kill-15 days			
Cr^{+6} $Na_2Cr_2O_7$	0.08 mg/liter	Significant mortality	Chinook salmon and *Salmo gairdneri*	Hardness: 70 mg/liter	Olson and Foster (1956)
Cr^{+6}	195 mg/liter	48 h TL_m	*Micropterus salmoides*		Fromm and Schiffman (1958)
	$\leq$ 20 mg/liter	Not lethal			
Cr ?	1.0 mg/liter	Acute toxic limit	*Gasterosteus aculeatus*		Hawksley (1967)
Cr^{+6}: $K_2Cr_2O_7$ K_2CrO_4	113 mg/liter 170 mg/liter	96 h TL_m	*Lepomis machrochirus*		Trama and Benoit (1960)
		96 h TL_m:			
$CrKSO_4$	5.07 mg/liter	Soft water	*Pimephales promelas*		Pickering and Henderson (1965)
	67.4 mg.liter	Hard water			
	7.46 mg/liter	Soft water	*Lepomis macrochirus*		
	71.9 mg/liter	Hard water			
	4.10 mg/liter	Soft water	*Carassius auratus*		
	3.33 mg/liter	Soft water	*Lebistes reticulatus*		
Cr^{+6}: $K_2Cr_2O_7$	17.6 mg/liter	Soft water	*Pimephales promelas*		
	27.3 mg/liter	Hard water			
	118.0 mg/liter	Soft water	*Lepomis macrochirus*		
	133.0 mg/liter	Hard water			

TABLE D-2. SUMMARY OF WORK DONE TO DETERMINE THE SUBLETHAL EFFECTS OF CHROMIUM ON ORGANISMS

Form	Concentration	Effect	Organism	Other	Reference
Cr ?	0.32-1.6 mg/liter	56 days, inhibits algal growth	*Lepocinclis steinii*		Hervey (1949)
	6.4-16.0 mg/liter	56 days, inhibits algal growth	*Chlorella variegatus*		
Cr^{+6}; CrO_4	1.39 mg/liter	Drastic reduction in production	Algae	Freshwater	Garton (1972)
	0.139 mg.liter	Slight (but significant) decrease in production			
Cr^{+6}	5.0 mg/liter	50% reduction in photosyntheses (4 days)	*Macrocystis pyrifera*	Salt water	Clendenning and North (1960)
Cr ?	?	Decreased respiration and activity due to reduced microbial reproduction	Sewage sludge		Ingols and Fetner (1961)
Cr^{+6}	10 mg/liter	10% reduction in BOD in 1 day	Sewage sludge		Heukelekian and Gillman (1955)
	100 mg/liter	50-90% BOD reduction			
Cr^{+3}	12.5 mg/liter 17.5 mg/liter 100 mg/liter	10% BOD reduction 50% BOD reduction 90% BOD reduction			

Continued

TABLE D-2. Continued

Form	Concentration	Effect	Organism	Other	Reference
Cr^{+3}: $CrCl_36H_2O$	0.6 mg/liter	50% reproductive impairment	*Daphnia magna*	Hardness: 45 mg/liter	Biesinger and Christensen (1972)
	0.33 mg/liter	16% reproductive impairment (maximum safe concentration)			
Cr^{+6}	0.0125 mg/liter	48% fewer offspring	*Neanthes arenaceodentata*		Southern California Coastal Water Research Project (1976)
Cr^{+6}: $Na_2Cr_2O_7$	0.2–0.4 mg/liter	Maximum safe concentration	*Salvelinus fontinalis*	Hardness: 45 mg/liter	Benoit (personal communication) Beisinger and Christensen (1972)
Cr^{+6}	2–4 mg/liter	Changes in blood, internal, or intracellular effects	*Salmo gairdneri*		Schiffman and Fromm (1959)
Cr^{+6}: $Na_2Cr_2O_7$	$\geq$0.016 mg/liter	Retarded growth rates	Chinook salmon	Hardness: 70 mg/liter	Olson and Foster (1956)
	$\geq$0.013 mg/liter	Retarded growth rates	*Salmo gairdneri*		
Cr^{+6}: CrO_4	31.0 mg/liter	No kill in 96 h	*Salmo gairdneri*		Garton (1972)
Cr^{+6}	sublethal	Reduced O_2 consumption; pathological changes in gut	*Micropterus salmoides*		Fromm and Schiffman (1958)

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APPENDIX E

WET WEIGHT-DRY WEIGHT RELATIONSHIP

To determine the dry weight of each dissected crayfish, the dry weights for all dissected tissues and the carcass had to be determined separately then summed. The error in these values is greater than that of intact body dry weights due to possible loss of body fluids and tissue during dissection and to statistical compounding of the error. For this reason, wet weight was used whenever a whole-body weight was needed in analysis. This occurred when total chromium body burdens in the laboratory feeding experiment was measured (expressed as ppb wet weight of chromium). Wet weights were also used in the determination of weight-independent metabolic rates. Dry weights of crayfish tissues and leaf material were easily and more accurately determined, therefore all metal concentrations for these samples are expressed as ppm dry weight.

Samples using the different weight expressions can be compared if wet weight:dry weight ratios are used to convert to common units. The relationship for the crayfish in both experiments is shown in Table E-1. There is little difference in the regression equations for males and females in the field experiment, thus a pooled regression for both sexes is sufficient. The equation for the crayfish in the laboratory experiment is quite different, however, so those data should be treated separately.

By substituting the measured wet weight value into the appropriate equation, dry weight can be obtained. These dry weights may then be used to obtain dry weight metal concentrations or metabolic rates. However, since treatments were pooled to obtain the regression equations, there is some risk in comparing the values for different treatments. If the regression equations are not the same for all treatments, comparisons would be distorted.

TABLE E-1. REGRESSION EQUATIONS, COEFFICIENTS OF DETERMINATION (r^2), AND SAMPLES SIZES FOR THE RELATIONSHIPS BETWEEN WET WEIGHT AND DRY WEIGHT OF CRAYFISH USED IN EXPERIMENTS[+]

Crayfish group	Regression equation	r^2	n
Metabolic rate experiment			
Males	$Y = 0.271X + 0.126$	0.872	23
Females	$Y = 0.287X + 0.077$	0.949	22
Males + females	$Y = 0.277X + 0.109$	0.931	45
Crayfish dissected, chromium-feeding experiment	$Y = 0.204X + 0.045$	0.823	8

[+] Y = dry weight; X = wet weight.

APPENDIX F

ACCURACY OF UNIVERSITY OF WISCONSIN NUCLEAR REACTOR DATA

Table F-1 presents the metal concentrations obtained for the "blind" standards inserted with the samples analyzed by the University of Wisconsin-Madison Nuclear Reactor, along with the actual concentrations in the substance. It should be noted that only muscle and exoskeleton were analyzed by the reactor. The hepatopancreas was analyzed using facilities of the Soil Science Department. The discrepancies are rather large, but there is no apparent trend and most are within the same order of magnitude. All analyses compared crayfish groups for each tissue; comparisons were never made between tissues analyzed by different methods. Consequently, it was valid to make comparisons using relative differences between crayfish groups, even though the accuracy of the reactor standards was questionable.

Because each value is based on only one sample rather than being a mean of several, no attempt was made to correct the crayfish tissue values. Statistical comparisons between groups would not change even if corrections were made.

TABLE F-1. COMPARISON OF METAL CONCENTRATIONS IN STANDARDS ANALYZED BY THE UNIVERSITY OF WISCONSIN-MADISON NUCLEAR REACTOR WITH THE REPORTED ACTUAL CONCENTRATIONS OF THE STANDARDS. STANDARD DEVIATIONS ARE REPORTED FOR THE VALUES OBTAINED BY THE REACTOR LABORATORY AND FOR THE ACTUAL CONCENTRATIONS IS KNOWN.

Sample	Source	Metal Concentration(ppm)				
		Ba	Cr	Fe	Se	Zn
SO-4 standard	Reactor	875.5±48.3	46.71±0.98	16,740±182	+	317.3±11.8
	Koons and Helmke (1978)‡	722±1.9	75±5.2	23,500±1.3	+	93±2.7
Orchard leaves standard	Reactor	+	3.008±0.346	+	< 0.03	+
	NBS§	+	2.3	+	0.08	+
Liquid standard¶	Reactor	49.34±2.58	27.84±0.19	+	0.363±0.009	+
	actual¶	60	40	+	5.0	+

[+] No value reported for actual value of standard.

[‡] R.D. Koons and P.A. Helmke. Neutron Activation Analysis of Standard Soils. Soil Sci. Soc. Am. J., 42(2):237-240, 1978.

[§] U.S. National Bureau of Standards.

[¶] Prepared from solutions of known concentrations.